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CLINICAL, BIOCHEMICAL AND NEUROIMAGING STUDIES IN FAMILIAL ALZHEIMER'S DISEASE

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JOHN-CASIMIR JOSEPH MARIE IGNATIUS JANSSEN

BSc MBBS MRCP

INSTITUTE OF NEUROLOGY

UNIVERSITY COLLEGE LONDON

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ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

Abstract

This thesis investigates several aspects of familial Alzheimer disease (FAD). The specific results of the work undertaken for this thesis were: (1) The proportion of FAD accounted for by mutations in known genes was 68% in 31 FAD families; Those without mutations were ten years older and associated with *APOE* ε4; (2) The phenotype of *PSEN1* FAD was found to share an early age at onset and features broadly suggestive of AD. However the phenotype was broad and included spastic paraparesis in association with "cotton wool" plaques in *PSEN1* E280G; (3) Plasma amyloid β peptide was found to be a potentially useful biomarker of FAD with levels being elevated in mutations carriers compared with non-carriers, and levels in at risk subjects falling halfway between the groups; (4) Cerebral atrophy in early onset AD was 2·8% (95% CI 2·3–3·3) per year which rose by 0·32% per year (0·15–0·50); (5) Pre-symptomatic medial temporal lobe atrophy in FAD at risk family members who become symptomatic was demonstrated with MRI medial temporal lobe volumes 16.6% lower in patients than controls and a higher rate of atrophy in patients than controls; (6) Cerebral atrophy in FAD was shown to begin in the posterior cingulate, temporoparietal and medial temporal cortices in presymptomatic FAD patients, a finding in accordance with previous cross-sectional neuropathological studies; (7) By contrast the onset and progression of cerebral atrophy in non-AD familial dementia was shown to markedly different in an presymptomatic FTLD patient with focal left frontal lobe onset reflecting the different clinical presentation; (8) Cerebral atrophy in FAD was found not to progress significantly faster than sporadic AD despite a twenty year age difference between the groups.

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Abbreviations

- 9dof, Nine degrees of freedom
- A2M, α2 macroglobulin
- AAO, Age at onset
- AChEI, Acetylcholinesterase inhibitor
- AD, Alzheimer's disease
- ADRDA, Alzheimer's disease and related disorders association
- APLP, amyloid precursor like protein
- APOE, Apolipoprotein E
- APP, Amyloid precursor protein
- ASOH, Allele specific oligonucleotide hybridization
- Aβ, β-amyloid peptide
- BACE, β-site APP cleaving enzyme
- BBSI, Brain boundary shift integral
- BP, Blood pressure
- CAA, Cerebral amyloid angiopathy
- CDR, Clinical dementia rating
- CERAD, Consortium to establish a registry for Alzheimer's disease
- CSF, Cerebrospinal fluid
- CT, Computed tomography
- CWP, cotton wool plaques
- DRG, Dementia Research Group
- DSM-IV, Diagnostic and statistical manual of mental disorders – 4^{th} edition
- EC, Entorhinal cortex
- EOAD, Early onset AD
- FAD, Familial AD (in this thesis EOAD inherited as autosomal dominant trait)
- FTD, Frontotemporal dementia
- FTLD, Frontotemporal lobar degeneration
- LOAD, Late onset AD
- LRP, Low density lipoprotein related receptor
- MCI, Mild cognitive impairment
- MIDAS, Medical information display and analysis system

- MMSE, Mini-mental state examination
- MRI, Magnetic resonance imaging
- MTL, Medial temporal lobe
- NART, National adult reading test
- NCSTN, Nicastrin
- NFT, Neurofibrillary tangle
- NIA, National institute for aging
- NINCDS, National institute of neurological and communicative disorders
- PA, Progressive non fluent aphasia
- PCR, Polymerase chain reaction
- PET, Positron emission tomography
- PHF, paired helical filaments
- PSEN1, Presenilin 1
- PSEN2, Presenilin 2
- ROI, Region of interest
- SAME, Subacute meningoencephalitis
- SD, Standard deviation
- SNP, single nucleotide polymorphism
- T, Tesla
- TE, Time to echo
- TIV, Total intracranial volume
- TR, Time to repeat
- VCM, Voxel compression map(ping)
- WAIS-R, Wechsler Adult Intelligence Scale - Revised

The problem

Alzheimer's disease (AD) is the commonest form of dementia. AD inevitably follows a progressive course. As age is the single biggest risk factor for the development of AD, rising life expectancy in the western world means that AD will increasingly impact not only on individuals and families, but also on society as a whole.

The aetiology of sporadic AD (SAD) is thought to result from a combination of environmental and genetic factors. By contrast autosomal dominant familial AD (FAD) is a rare genetic form of the disease that occurs in the presenium. Patients from families with FAD particularly lend themselves to clinical, biochemical and neuroimaging studies as they generally do not have the co-morbidities seen in SAD occurring in later age. Furthermore the development of symptoms and signs can reasonably be taken to be due to FAD even without neuropathological confirmation. However, the Dementia Research Group (DRG) collection of FAD families contained a number of families that had previously been screened without success for mutations in the recognised causative FAD genes: *APP*, *PSEN1* and *PSEN2*, all of which affect APP processing and alter amyloid β production - a key component of neuritic plaques. The study of the genetics of these families allowed the determination of the proportion accounted for by known FAD genes, and the clinical phenotype associated with these mutations. Since amyloid β peptide is not only synthesised in brain, but also in platelets the assessment of plasma amyloid β peptide as a biomarker of FAD in family members could also be performed.

The pathological signature lesions of AD are extra-cellular amyloid plaques, and intracellular neurofibrillary tangles. It is not yet possible to assess the distribution or quantity of these abnormal proteins reliably in vivo. However an inevitable effect of neuronal loss is cerebral atrophy. This atrophy is seen at post-mortem, but may also be visualized *in vivo* using volumetric magnetic resonance imaging (MRI). In AD, atrophy is both global, as well as regional, with early and prominent involvement of medial temporal lobe structures. In order to quantify rates of brain atrophy at an individual level, it is necessary to compare serial scans taken from the same individual over time. In order to facilitate accurate comparison, these scans can be digitally matched (registered) to one another, with a high degree of precision. It is then possible to measure rates of global atrophy either by comparing the brain volumes directly, by assessing expansion of cerebrospinal fluid space over time, or by using an

automated method of brain volume change, the brain boundary shift integral (BBSI). Atrophy rates derived using the BBSI can distinguish groups of patients with Alzheimer's disease from controls over a one year period. Using these imaging techniques the rate of progression of AD over a period of many years can be established and whether this process accelerates; the medial temporal lobe, the proposed area of earliest change, can be studied in at risk FAD patients during the time that they become affected to determine whether there is pre-symptomatic atrophy; furthermore using fluid registration (where the brain is treated as a viscous fluid rather than a rigid body) of serial MRI the site of earliest change can be determined *in vivo* without *a priori* decisions based on established neuroimaging and neuropathological knowledge. Having established the onset and progression of cerebral atrophy in FAD this can be compared with non-AD familial dementia to see whether this pattern is the same or, like the clinical presentation, different potentially adding to the biological plausibility of the technique. The progression of cerebral atrophy in FAD has not previously been compared with SAD and the Specialist Cognitive Disorders Clinic at the National Hospital for Neurology and Neurosurgery proved a valuable source of patients to allow this comparison.

The aim of the work undertaken for this thesis was to determine whether there were any novel FAD mutations in DRG families, whether these were associated with unusual phenotypes, and whether plasma amyloid β peptide was a useful biomarker. Neuroimaging studies using MRI were performed to assess how cerebral atrophy starts and how it progresses over time in FAD. I had the opportunity of comparing this with the onset of cerebral atrophy in non-AD familial dementia. I also had the opportunity of comparing the progression of cerebral atrophy of FAD with SAD.

Specific aims were: (1) to determine the proportion of FAD accounted for by mutations in known genes; (2) to determine whether there is a specific *PSEN1* FAD phenotype; (3) to determine whether plasma amyloid β peptide is a useful biomarker of FAD; (4) to determine whether the progression of cerebral atrophy in early onset AD is linear; (5) to determine whether there is pre-symptomatic medial temporal lobe atrophy in FAD at risk family members who become symptomatic; (6) to determine where cerebral atrophy in FAD begins in at risk family members who become symptomatic; (7) to assess whether the onset and progression of cerebral atrophy is different in non-AD familial dementia; (8) to compare cerebral atrophy in FAD and SAD to determine whether FAD progresses more quickly.

Declaration

The data and studies reported in this thesis were performed as part of an ongoing large collaborative study funded by the Medical Research Council (programme grant number G9626876) that involves many clinicians and researchers. Work performed in collaboration with others, or performed by others as part of a study is acknowledged in the individual data chapters and in outline in the acknowledgements at the end of this thesis.

My personal contribution includes: review of the DRG families; recruitment of patients and several new families; scheduling of all patient assessments; obtaining informed consent; arranging all MRI scans and all neuropsychology assessments; clinical assessment of all patients and the majority of control subjects, which included history and neurological examination; venepuncture of all patients; preparation, storage and transport of all blood and plasma specimens; brain donation consent; liaison with patients' general medical practitioners and/or hospital specialists when indicated; review of medical records; individual study concepts and design; MRI scan quality review; some whole brain segmentations; some brain registrations; all ventricular segmentations; statistical analyses; data interpretation.

1. Introduction

1.1 Dementia

Dementia is a clinical syndrome characterised by a decline in both cognitive and social function to the extent that it interferes with the activities of daily living. A concise definition is provided by the American Psychiatric Association Diagnostic and Statistical Manual of Mental Disorders version 4 (DSM-IV) (1994). This requires the development of multiple cognitive deficits that include memory impairment and at least one of: aphasia (language disturbance), apraxia (impaired ability to carry out motor activities despite intact motor function), agnosia (failure to recognise or identify objects despite intact sensory function) or executive dysfunction (i.e. disturbance in planning, organising, sequencing, abstracting). A diagnosis of dementia should not be made if the cognitive deficits occur exclusively in the presence of delirium. Usually the disorder is progressive, but occasionally may be reversed or arrested when the underlying aetiology is treatable. Not uncommonly patients develop, or occasionally present with, depression, personality change or apathy.

The prevalence of dementia depends on the criteria used to define the syndrome and can vary tenfold (Erkinjuntti *et al.* 1997). Regardless of the criteria used there is a clear age related increase in incidence of dementia. Meta-analyses of studies done in developed countries have established dementia prevalence at around 1.5% at age 65 years, doubling every four years to reach about 30% at 80 years (Ritchie & Lovestone 2002). Dementia is currently the third most common cause of death and is predominantly a problem that affects the developed world. However, the larger population size and increasing longevity in the developing world means that the number of patients with dementia is rising faster than at any time in history and it is estimated that it will be the second most common cause of death by the middle of this century. There are many causes for the syndrome of dementia, the most common being Alzheimer's disease (Ritchie & Lovestone 2002).

1.2 Alzheimer's disease

In 1907 Alois Alzheimer reported the case of Auguste D, a 51 year old woman who had developed multiple cortical deficits with prominent dysphasia, dyslexia, dysgraphia, and agnosia in addition to a severe memory impairment and paranoid delusions . Neuropathological examination of her brain using the then newly available

silver stains revealed senile plaques, consisting of dystrophic neurites, and dense perikaryal staining, which was termed the neurofibrillary tangle. The cerebral cortex was atrophic, which was assumed to be due to cell loss. Alzheimer subsequently wondered how senile dementia differed from normal ageing, and whether cognitive impairment seen in the elderly should be considered a separate entity from the presenile cases that he had already reported. Specific clinical features, in particular dysphasia, were more prominent in younger onset patients and Emil Kraepelin concluded that there was a difference and proposed the eponym Alzheimer's disease. The disease continued to be considered a rarity confined to the presenium until the landmark studies of Blessed, Tomlinson and Roth (1968), who showed that the neuropathological substrates of senile dementia and Alzheimer's disease (AD) were identical, making AD one of the most common diseases of an ageing population.

1.2.1 Epidemiology

1.2.1.1 Prevalence

There are an estimated 400,000 patients with AD in the United Kingdom (Alzheimer Society (2004), UK) and nearly five million in the United States of America (Hebert *et al.* 2003). The prevalence of AD increases rapidly with age from 0.3% in the seventh decade of life, to 3.2% in the eight decade of life, and to 10.8% in the ninth decade of life (Kokmen *et al.* 1989; Rocca *et al.* 1991). In view of the improved survival of those aged over 75 years, the number of AD patients in the USA is expected to triple to 13.2 million by 2050 with a concomitant increase in the economic and caregiver burden (Hebert *et al.* 2003).

1.2.1.2 Risk factors

As discussed above Alzheimer's disease is increasingly common in late life and age appears to be the primary risk factor for AD. Familial clustering is recognised in 10% of AD patients and family history is the most important risk factor after age (Van Duijn *et al.* 1991). Some families display an autosomal dominant pattern of inheritance, a fact which was recognised relatively soon after Alzheimer's original description of Auguste D (Lowenberg & Waggoner 1934; Schottky 1932). The genetics of AD, the role of Apolipoprotein E (APOE) genotype and autosomal

dominant AD genes will be discussed in detail later. Other associations such as head trauma are less robust (Mayeux *et al.* 1993; Mehta *et al.* 1999; van Duijn *et al.* 1992).

Recently there has been much interest in the association of atherosclerosis and "vascular" risk factors with the development of AD (Snowdon *et al.* 1997). A Dutch prospective incidence study from Rotterdam showed a significant relationship between vascular risk factors and AD (Kalmijn *et al.* 1997; Ott *et al.* 1997; Ott *et al.* 1998; Ott *et al.* 1999). In this three year longitudinal study of 6000 residents from a specific district of Rotterdam 126 residents developed dementia of whom 89 were diagnosed with AD. Atherosclerosis, cigarette smoking, hypercholesterolaemia, cardiovascular disease and diabetes were all associated with a significantly higher risk of AD. There was a doubling of risk associated with type II diabetes mellitus, the risk being greatest for those who were insulin requiring. Atrial fibrillation was a greater risk factor for AD than vascular dementia in this study, which may reflect the relative incidences of these two dementias. Smoking and a high fat diet were independent risk factors that also doubled risk, although when diet was looked at in more detail a high intake of total, saturated, and trans fat and cholesterol (and low intake of mono or poly unsaturated fats) were not associated with increased risk of dementia or its subtypes (Engelhart *et al.* 2002). Moderate alcohol consumption appeared to be protective (Ruitenberg *et al.* 2002), a finding previously demonstrated in the French PAQUID cohort, an epidemiological study of normal and pathological aging after 65 years of 2950 non-demented people living at home in the south west of France (Commenges *et al.* 2000).

A Finnish epidemiological study showed that patients with a raised systolic blood pressure (BP) or high serum cholesterol in midlife had a significantly higher risk of AD in later life, and their effect appeared to be additive (Kivipelto *et al.* 2001). However, the data on blood pressure are complex. Studies that report an association between hypertension and AD share a long observation period before onset of dementia. High systolic or diastolic BP at 70 years of age was predictive of onset of AD between the ages of 79 and 85 years in the Gothenburg Longitudinal Study on Aging (Skoog *et al.* 1996). Similarly systolic BP above 160 mmHg or diastolic BP above 90 mmHg measured between 1961 and 1965 was predictive of AD diagnosis in 1991 in the Honolulu-Asia Aging study (Launer *et al.* 2000). Longitudinal studies therefore report associations between early hypertension, but not

concurrent hypertension and dementia. Cross sectional studies and short term longitudinal studies report associations between low BP and dementia (Ruitenberg *et al.* 2001). This may be because BP declines in the years preceding the onset of AD and that BP continues to decline during the course of the disease. A more recent study suggests that both very low diastolic BP (<66 mmHg) and very high systolic BP (>180 mmHg) were related to an increased incidence of AD in the six year Kungsholmen study (Qiu *et al.* 2003). This increased risk of AD due to low diastolic BP was only found in those treated with antihypertensive drugs, which suggests that patients at high risk of AD *had* a history of hypertension.

In population terms vascular risk factors are potentially very important as some of them may be modifiable. Supporting evidence of vascular risk factor assessment and appropriate treatment is now beginning to emerge (in't Veld *et al.* 2001). The reduction of cholesterol seems to be particulary beneficial with a report of a 70% reduction in the incidence of AD (Jick *et al.* 2000) and a 39% lower risk of AD in statin users relative to nonstatin users (Zamrini, McGwin, & Roseman 2004). The epidemiological data are underpinned by multiple laboratory studies. A reduction in cholesterol levels results in both a reduction in plasma Aβ peptide levels and Aβ deposition in brain (Fassbender *et al.* 2001; Kojro *et al.* 2001; Refolo *et al.* 2001). In the study by Kojro *et al.* (2001) cholesterol lowering treatment of peripheral and neural cell lines resulted in a dramatic increase of secreted α-secretase cleaved soluble APP, and an accompanied reduction in secretion of Aβ peptides. Similar results were obtained in mice by Refolo *et al.* (2001) who additionally observed a strong, positive correlation between the amount of plasma cholesterol and Aβ. The mechanism of action of statins in the prevention of AD is unclear, but it seems that this is an effect beyond cholesterol lowering (for review see Crisby *et al.* 2002). Clearly this opens a novel and relatively safe therapeutic pathway for primary and secondary prevention and possibly treatment of AD.

1.2.2 Clinical features

Alzheimer's disease is characterised by progressive memory impairment of insidious onset. Episodic or autobiographical memory is primarily affected with the early loss of event memory. Frequently patients have difficulty remembering familiar routes. Procedural memory and short term memory, as in digit span, are preserved until later

in the illness. Semantic memory problems, attentional deficits and executive dysfunction typically appear after the initial amnestic symptoms.

A collateral history from the spouse or other informant is essential as the patient may not be aware of the presence or severity of these deficits (anosognosia) and is often capable of maintaining a social façade that can mask the cognitive impairment. Deficits in problem solving, language, calculation and visuospatial skills appear relatively early in the disease and reflect dysfunction of non-memory cognitive domains. Atypical forms of AD are well recognised and include progressive visual dysfunction, progressive biparietal dysfunction, and progressive aphasia (for review see Galton *et al.* 2000).

Non-cognitive symptoms occur frequently: depressed mood can occur prior to or coincident with diagnosis in up to 25% of patients (Devanand *et al.* 1996). Delusions, hallucinations and aggression are a frequent management problem later on and are often the most distressing to patients and family members (Burns, Jacoby, & Levy 1990; Harvey 1996). These behavioural features are common reasons for institutionalisation.

Early in the disease abnormalities on neurological examination are usually confined to deficits of higher mental function. Dyspraxia is typical once patients are moderately affected. Gegenhalten may be found (Frommelt *et al.* 1991; Tyrrell & Rossor 1988), but the presence of extrapyramidal features in the absence of neuroleptic treatment raises the possibility of a diagnosis of dementia with Lewy bodies (Lippa, Smith, & Swearer 1994; McKeith *et al.* 1994) or possibly frontotemporal lobar degeneration (Foster *et al.* 1997; Neary *et al.* 1998).

Motor function and co-ordination are typically preserved until late in the disease. Seizures are a recognised late manifestation and may be predated by myoclonus, with some evidence that both these features appear more frequently in early onset AD (Forstl *et al.* 1992; Janssen *et al.* 2000; Mayeux, Stern, & Spanton 1985).

After a gradual decline all testable cognitive function is lost, and the patient becomes mute, bedbound and usually succumbs to intercurrent medical complications. From the time of diagnosis, the disease runs approximately four to 16 years until the time of death with a mean survival of ten years (Jost & Grossberg

1995). In recent times survival has been increasing, though this may in part reflect earlier diagnosis and the increasing realisation that AD may be associated with a prolonged amnestic prodrome (Backman, Small, & Fratiglioni 2001; Elias *et al.* 2000). This relatively subtle cognitive dysfunction has generated much work to define the syndrome of (amnestic) mild cognitive impairment (MCI), which may represent a precursor state of AD (Petersen *et al.* 1999).

Whilst AD has a devastating effect on the patient, in certain respects it takes an even greater toll on their family. Family care givers commonly feel helpless, frustrated, exhausted and often face considerable financial difficulties. Almost half of family care givers become depressed (Cohen & Eisdorfer 1988). Although medical guidance and support can help them anticipate and prepare for some of the consequences of AD, family members frequently find themselves reacting to crises as they arise. As a result dementia care requires a multidisciplinary approach with input from many professionals and the voluntary sector.

1.2.3 Diagnostic clinical criteria

A definitive diagnosis of AD requires neuropathological examination of the brain to demonstrate neuritic plaques and neurofibrillary tangles, the hallmark lesions of AD. However, over the last fifteen years the reliability of ante mortem diagnosis of AD has improved significantly. This may in part reflect the increased interest in AD resulting from the development and licensing of the acetylcholinesterase inhibitors (AChEI), donepezil, rivastigmine and galantamine (Rogers *et al.* 1998; Rosler *et al.* 1999; Wilcock, Lilienfeld, & Gaens 2000) and more recently the N-Methyl-D-aspartate (NMDA) type receptor antagonist memantine (Doody *et al.* 2004), but is largely due to the development of standardised diagnostic criteria. The most prominent amongst these are the research criteria developed jointly between the National Institute of Neurological and Communicative Disorders and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) (McKhann *et al.* 1984). The NINCDS-ADRDA criteria define diagnoses of "definite", "probable", and "possible" AD. The NINCDS-ADRDA criteria were used in the studies described in this thesis and unless otherwise specified AD refers to "probable" AD. The sensitivity and specificity of the NINCDS-ADRDA criteria discussed below have been found to

be relatively independent of the specific criteria used for a neuropathological diagnosis of AD (Nagy *et al.* 1998).

Definite AD is reserved for the gold standard of histopathological examination of the brain at autopsy. Brain biopsy provides a lesser degree of diagnostic certainty but is included in this category. Probable AD requires the presence of dementia to be established by clinical examination and simple cognitive tests, and confirmed by neuropsychological tests. The symptoms of memory loss and other cognitive deficits must be progressive. A Probable AD diagnosis also requires the absence of disturbances of consciousness, other brain diseases and systemic illnesses that could explain the cognitive impairments. Impaired activities of daily living and a positive family history of AD are considered supportive of the diagnosis. The latter may allow the inclusion of patients with autosomal dominant AD with an age at onset younger than the minimum age of 40 years considered to be the threshold in the criteria. The diagnosis of Probable AD is considered unlikely when the onset is sudden and/or certain focal deficits are present, as distinct from the gradual and insidious decline expected in AD; seizures or extrapyramidal symptoms occurring early in the course of the disease would make the Probable AD diagnosis unlikely. Possible AD is used to describe those cases of dementia (or progressive isolated cognitive impairments) in which the onset, presentation, or clinical course is somewhat atypical, yet no other explanation for the impairment is found. In the event that a systemic or other brain disease is present the diagnosis of possible AD can still be applied providing the second illness is not considered to be the cause of the dementia. The criteria are listed in full below.

1.2.3.1 NINCDS-ADRDA criteria for Alzheimer's disease

I. Criteria for the diagnosis of <u>Definite</u> Alzheimer's disease:

- Clinical criteria for probable Alzheimer's disease fulfilled, AND
- Histopathological evidence obtained from autopsy

II. Criteria for the diagnosis of <u>Probable</u> Alzheimer's disease:

- Dementia established by clinical examination, and documented by the MMSE, Blessed Dementia scale or some similar examination, and confirmed by neuropsychological tests
- Deficits in two or more areas of cognition
- Progressive worsening of memory and other cognitive functions

- No disturbance of consciousness
- Onset between ages 40 and 90, most often after age 65
- Absence of systemic disorders or other brain diseases that in and of themselves could account for the progressive deficits in memory and cognition

III. Diagnosis of <u>Probable</u> Alzheimer's disease is supported by:

- Progressive deterioration of specific cognitive functions such as language (aphasia), motor skills (apraxia) and perception (agnosia)
- Impaired activities of daily living and altered patterns of behaviour
- Family history of similar disorders, particularly if confirmed neuropathologically
- Normal lumbar puncture as evaluated by standard techniques
- Normal pattern or non-specific changes in EEG, such as increased slow-wave activity
- Evidence of cerebral atrophy on CT and progression documented by serial observation

IV. Other clinical features consistent with the diagnosis of <u>Probable</u> Alzheimer's disease, after exclusion of causes of dementia other than Alzheimer's disease, include:

- Plateaus in the course of progression of the illness
- Associated symptoms of depression, insomnia, incontinence, delusions, illusions, hallucinations, catastrophic verbal, emotional, or physical outbursts, sexual disorders and weight loss
- Other neurological abnormalities in some patients especially with more advanced disease and including motor signs such as increased muscle tone, myoclonus or gait disorder
- Seizures in advanced disease
- CT normal for age

V. Criteria that make a diagnosis of <u>Probable</u> Alzheimer's disease uncertain or unlikely include:

- Sudden, apoplectic onset
- Focal neurological findings such as hemiparesis, sensory loss, visual field deficits, and incoordination early in the course of the illness
- Seizures or gait disturbance at the onset or very early in the course of the illness

VI. Clinical diagnosis of <u>Possible</u> Alzheimer's disease:

- May be made on the basis of the dementia syndrome, in the absence of other neurological, psychiatric, or systemic disorders sufficient to cause dementia, and in the presence of variations in the onset, in the presentation or in the clinical course

- May be made in the presence of a second systemic or brain disorder sufficient to produce dementia, which is not considered to be *the* cause of dementia
- Should be used in research studies if the presence of a single gradually progressive severe cognitive deficit is identified in the absence of other identifiable cause

1.2.3.2 Accuracy of NINCDS-ADRDA Diagnostic Criteria

In order to reach a specific dementia diagnosis, the physician integrates information from patient and caregiver interviews, medical, neurological and psychiatric examinations, and the results of investigations. The entire dementia evaluation process is often treated as though it is a single diagnostic test for the purpose of defining diagnostic sensitivity, specificity and predictive values.

Relative to autopsy, the Possible AD category is associated with low sensitivity (47-56%) and low specificity (43-61%), while the Probable AD category has excellent specificity (97-100%) but low sensitivity (34-47%) (Nagy *et al.* 1998). The combination of the Probable and Possible AD categories results in a high sensitivity, ranging from 91-98%, but the specificity of the criteria remains relatively low, ranging from 23-61% (Jobst, Barnetson, & Shepstone 1998; Nagy *et al.* 1998; Varma *et al.* 1999).

By definition, the distinction between Probable and Possible AD diagnoses is the degree to which comorbid confounders are present and the extent to which the symptoms and/or progression are atypical. The high specificity of a Probable AD diagnosis reflects the fact that AD can be reliably diagnosed when presenting in the classical amnestic manner. However, the poor sensitivity of the Probable AD category indicates that many AD patients do not present in this way. Alzheimer's disease may be difficult to distinguish from other forms of dementia when other conditions are present or the course is atypical. A factor which is rarely considered in assessing the accuracy of an AD diagnosis is disease severity at presentation. The clinical phenotypes of different forms of dementia tend to be most evident early in the disease process. The trend towards earlier diagnosis of AD, however, creates a potential for misdiagnosis partly due to the subtlety of symptoms and partly because of the limited history of progression available in early stage patients and patients with MCI.

1.2.4 Genes associated with Alzheimer's disease

A genetic component to AD has been postulated since the recognition of familial cases in the 1930's (Lowenberg & Waggoner 1934; Schottky 1932), and a family history is considered a supportive feature in the NINCDS-ADRDA diagnostic criteria. As discussed later in this thesis many families with multiple affected members have been reported, and in the majority of these the mode of inheritance appears to be autosomal dominant with an age at onset in the presenium. The latter may be a confounding factor in that familial clustering will be easier to recognise and the genetic patterns in these families may not be typical. Many diseases for which there is a genetic basis require specific environmental conditions to express the disease. If a gene has an allelic variant that is, more often than not, found among patients with a specific disease, then that gene is said to be associated with the disease. Not everyone possessing the high susceptibility allele develops the disease, and some, perhaps many, will also become affected for other reasons. It is often difficult to determine whether the associated gene is pathogenic or merely a surrogate marker for another genetic risk factor. These AD associated genes are termed risk genes and they are discussed in this section.

1.2.4.1 Apolipoprotein E gene

Good evidence now exists that Apolipoprotein E (ApoE, protein; *APOE*, gene) is an important contributor to AD susceptibilitly. Apolipoprotein E is a plasma protein involved in cholesterol transport encoded by the *APOE* gene on chromosome 19q13.2 (Zannis, Kardassis, & Zanni 1993). Though ApoE is primarily produced in the liver, it is also synthesised by astrocytes in the central nervous system and intimately involved in the growth and repair of the nervous system during development or after injury (Ignatius *et al.* 1986; Pitas *et al.* 1987). ApoE is increased in several chronic neurodegenerative diseases including AD and Creutzfeldt Jakob disease (Namba *et al.* 1991). Antisera to ApoE stain senile plaques, neurofibrillary tangles and cerebral vessel amyloid deposits (Namba *et al.* 1991; Strittmatter *et al.* 1993; Wisniewski & Frangione 1992).

There are three common allelic variants designated *APOE-ε2, -ε3* and *-ε4*. Because everyone inherits one *APOE* allele from each parent, there are six possible *APOE* genotypes: *APOE-ε2/2, APOE-ε2/3, APOE-ε2/4, APOE-ε3/3, APOE-ε3/4,* and

APOE-ε4/4. These variants result in amino acid substitutions (Arg and Cys) at positions 112 and/ or 158 of the isoprotein (Zannis, Kardassis, & Zanni 1993). In Caucasians the allele frequencies are *ε2* = 0.08, *ε3* = 0.77 and *ε4* = 0.15 (Campion *et al.* 1999; Weiner *et al.* 1999; Zannis, Kardassis, & Zanni 1993).

It is now well accepted that the frequency of the ε4 allele of *APOE* is overrepresented in patients with AD. This observation was initially made in late onset familial AD (Strittmatter *et al.* 1993). It was rapidly replicated and established that sporadic late onset as well as young onset cases had approximately a threefold higher than expected likelihood of having *APOE-ε4* (Poirier *et al.* 1993; Rebeck *et al.* 1993; Saunders *et al.* 1993). These findings suggest that inheritance of *APOE-ε4* is a risk factor in the development of AD. In addition, early studies found that *APOE-ε2* was underrepresented in AD, suggesting that it is a negative or protective risk factor for AD (Corder *et al.* 1994; West, Rebeck, & Hyman 1994). Corder and colleagues (1994) initially estimated that in late onset familial AD the *APOE-ε4* associated risk for dementia approached 100% by the age of 80 years. However, this observation was derived from a highly selected patient group with a very strong family history of AD. Since that time data from several population-based studies have demonstrated that the risk of developing AD for an individual in the general population is much lower than it initially appeared with a range in odds ratios associated with *APOE-ε4*, from 1.4 (Iowa study) to 3.7 (Framingham study) (Feskens *et al.* 1994; Hyman *et al.* 1996; Kuusisto *et al.* 1994; Myers *et al.* 1996). The differences in risk ratios among different studies might in part be due to differences in study design, population differences, and differences in age and gender distribution in those populations.

These same studies have also shown that the average age at onset of AD patients who carry one or two copies of the *ε4* allele is significantly lower than that of patients who did not inherit this allele. In a study of a clinic based population of 359 patients with AD, the age at onset of *APOE-ε4/ε4* patients was 67.1 ± 7.6 (mean ± SD) years, five years earlier than *APOE-ε3/ε4* patients at 72.3 ± 8.3 years, and seven years earlier than *APOE-ε3/ε3* patients at 73.9 ± 11.7 years (Gomez-Isla *et al.* 1996b). Thus AD patients with one or two *ε4* alleles do have an earlier age at onset, with a dose effect, a finding that has been replicated on several occasions (Blacker *et al.* 1997; Meyer *et al.* 1998). However, as illustrated above, these ages at onset are broadly overlapping among *APOE* genotypes, and the genotype-age relationship is not applicable to an individual patient.

1.2.4.2 Other risk genes

Although *APOE-ε4* is the risk gene that has the most robust association with AD it is neither sufficient nor necessary for the development of AD. Relatives of AD patients without an *APOE* ε4 allele have been found to be at increased risk of AD, suggesting that other genes or risk factors may be involved in the pathogenesis of AD (Devi *et al.* 1999).

In 1998 two case control studies (Blacker *et al.* 1998; Liao *et al.* 1998) reported an association between the α_2-macroglobulin gene *(A2M)* on chromosome 12 and late onset AD, whereas others have not been able to replicate this finding (Wavrant-DeVrieze *et al.* 1999). In a Finnish prospective population based clinicopathological study of late onset AD, the *A2M* exon 24 A/A genotype was associated with a diagnosis of Definite AD, but not with Probable AD (Myllykangas *et al.* 1999). No association was found with the other *A2M* polymorphism (5 base pair deletion upstream of exon 18), an observation confirmed in a study from Taiwan (Hu *et al.* 1999).

α_2-macroglobulin is an abundant serum protease inhibitor and a major ligand of the low density lipoprotein related receptor protein (LRP). LRP is found in neuritic plaques. It binds cholesterol, several other AD related ligands, and is also the primary ApoE receptor in neurons. Like *A2M,* the *LRP* gene is also located on chromosome 12 and it may itself be a risk factor for AD affecting primarily neuritic plaque development (Kang *et al.* 1997). A meta-analysis found the *LRP-C* allele to be overrepresented in definite AD patients compared to control subjects (86.7% versus 82.8%; odds ratio 1.34; $P < 0.0001$) (Beffert, Arguin, & Poirier 1999). However, there appears to be no difference in age at onset for AD between *LRP* genotypes (Warwick Daw *et al.* 2000).

Linkage between chromosome 12 and AD has been demonstrated consistently, and since the *LRP* association is relatively weak, it has been suggested that another locus on chromosome 12 is a more likely candidate as a risk factor for AD, possibly at a site near to *A2M* (Martin, Martin, & Borgaonkar 1998; Rogaeva *et al.* 1998; Wu *et al.* 1998). This further chromosome 12 candidate gene maybe the transcriptional factor LBP-1c/CP2/LSF, which lies just 6cM proximal to *LRP* and regulates the expression of numerous genes including *A2M.* A protective association between a G/A polymorphism in the 3'- untranslated region of this gene and SAD has

been reported in French, British and North American populations (Lambert *et al.* 2000). This protective finding has been replicated in two further SAD series, the earlier of which was autopsy confirmed (Luedecking-Zimmer *et al.* 2003; Taylor *et al.* 2001). However, further studies will be necessary to reveal the real identity of this gene.

Recent papers provide evidence for an AD locus on chromosome 10 independent of *APOE* genotype (Ertekin-Taner *et al.* 2000; Myers *et al.* 2000). Interestingly the insulin degrading enzyme (IDE), which has a central role in the degradation and clearance of Aβ, also lies on chromosome 10 (Bertram *et al.* 2000; Vekrellis *et al.* 2000). Although IDE maps distal to the peak of linkage shown by Myers *et al.* (2000), Bertram *et al.* (2000) found linkage with four out of six markers close to the *IDE* locus. Furthermore physical mapping data have shown that *IDE* and its closest marker lie on the same bacterial artificial chromosome (GenBank accession number AL356128). Overall these findings indicate an AD susceptibility gene on the long arm of chromosome 10, although it may not be *IDE* (Bian *et al.* 2004; Boussaha *et al.* 2002).

1.3 Familial Alzheimer's disease

Population studies have shown that 25-62% of AD patients have at least one other affected first degree relative, the higher values being obtained in younger patients (Campion *et al.* 1999; van Duijn *et al.* 1994). In elucidating the genetic aetiology of AD it became clear that because of variations in AAO and transmission patterns in different families that there was clinical and genetic heterogeneity (Farrer *et al.* 1990). Farrer *et al.* (1990) analysed AAO in 70 familial AD kindreds and concluded that two or more mechanisms were involved in the expression of the illness. There was evidence for two distinct groups of families based on bimodality of AAO, with a cutoff age of 58 years. As a result two distinct categories of AD can be distinguished: pre-senile or early onset AD (EOAD) and senile or late onset AD (LOAD). Furthermore, in EOAD families the pattern of transmission is consistent with an autosomal dominant inheritance model. In some LOAD families the disease also segregates as an autosomal dominant trait, but the pattern in the majority of LOAD families is less clear suggesting that other genetic or shared environmental factors contribute. In view of EOAD families being clearly autosomal dominant the term familial AD (FAD) in this thesis refers to these families only unless further specified.

To determine the prevalence of FAD we first need to review the epidemiological literature of EOAD.

1.3.1 Epidemiology

Most of the epidemiological studies on AD have measured prevalence of the disease in elderly populations. Of these only three studies include estimates of the prevalence of EOAD (Kokmen *et al.* 1989; Schoenberg, Anderson, & Haerer 1985; Sulkava *et al.* 1985). A further four studies were specifically designed to determine the prevalence of early onset dementia (Harvey, Skelton-Robinson, & Rossor 2003; McGonigal *et al.* 1993; Newens *et al.* 1993; Ratnavalli *et al.* 2002), whereas only one study has looked specifically at the prevalence of FAD (Campion *et al.* 1999); these data are summarised in Table 1.1.

These population based studies varied in their design and diagnostic criteria. Schoenberg *et al.* (1985) used the diagnosis "severe dementia" of unspecified aetiology whereas Sulkava *et al.* (1985) used the diagnosis "primary degenerative dementia". The more recent studies have used standard diagnostic criteria. In the Framingham study, Kokmen *et al.* (1989) used NINCDS-ADRDA criteria. In the UK Northern Health Region Study Newens *et al.* (1993) used DSM-III-R criteria (American Psychiatric Association 1987). The NINCDS-ADRDA criteria were used in the Scotland Study (McGonigal *et al.* (1993)), the London Study (Harvey *et al.* (2003)) and the Cambridge Study (Ratnavalli *et al.* (2002)).

The only epidemiological study to determine the relative prevalence of EOAD and FAD performed to date is a population based study from the French city of Rouen (population 426,710) (Campion *et al.* 1999). In this study all known patients with a diagnosis of dementia with an AAO of less than 61 years were referred to a single neurology clinic by local general practitioners, neurologists, and psychiatrists. Nursing/residential home or psychiatric hospital in-patients were excluded if they had not been resident in Rouen prior to their institutionalisation. Only Probable AD (NINCDS-ADRDA) patients were included. To determine family history a structured questionnaire was used to obtain a detailed three generational family history from the next of kin and confirmed by at least a second informant. A mutational analysis of the three causative AD genes (see section 1.3.2) was performed in all familial patients. They identified 39 patients with EOAD; 15 were sporadic cases and 24 had a positive family history. Of those with a family history five (13%) were autosomal dominant

early onset AD (FAD) defined as having patients with early onset AD in each of three generations. Two further patients had a family history of *both early and late onset* within three generations (termed ADAD in their study) and 17 patients did not have a family history that corresponded with either FAD or ADAD. The calculated global prevalence per 100,000 inhabitants at risk, defined in this study as those aged 41-60 years, was 41.2 for early onset AD and 5.3 for FAD.

Table 1.1: Prevalence of early onset AD

Study (first author)	Age range (years)	Individuals at risk	Cases found	Prevalence (per 100,000)
Schoenberg 1985	40-59	4,422	2	45.2
Sulkava 1985	30-59	5,466	1	18.2
Kokmen 1989	45-59	7,422	2	26.9
Newens 1993	46-64	655,800	227	34.6
McGonigal 1993	40-64	1,402,655	317	22.6
Campion 1999	41-60	94,593	39	41.2
Ratnavalli 2002	45-64	72,815	11	15.1
Harvey 2003	30-64	193,493	42	21.7

1.3.2 Causative genes and pathogenic mutations

Three causative genes for FAD have been identified: the β amyloid precursor protein (*APP*; OMIM 104760) gene on chromosome 21 (Goate *et al.* 1991), the presenilin 1 (*PSEN1*; OMIM 104311) gene on chromosome 14 (Sherrington *et al.* 1995m) and the presenilin 2 (*PSEN2;* OMIM 600759) gene on chromosome 1 (Levy Lahad *et al.* 1995b; Rogaev *et al.* 1995).

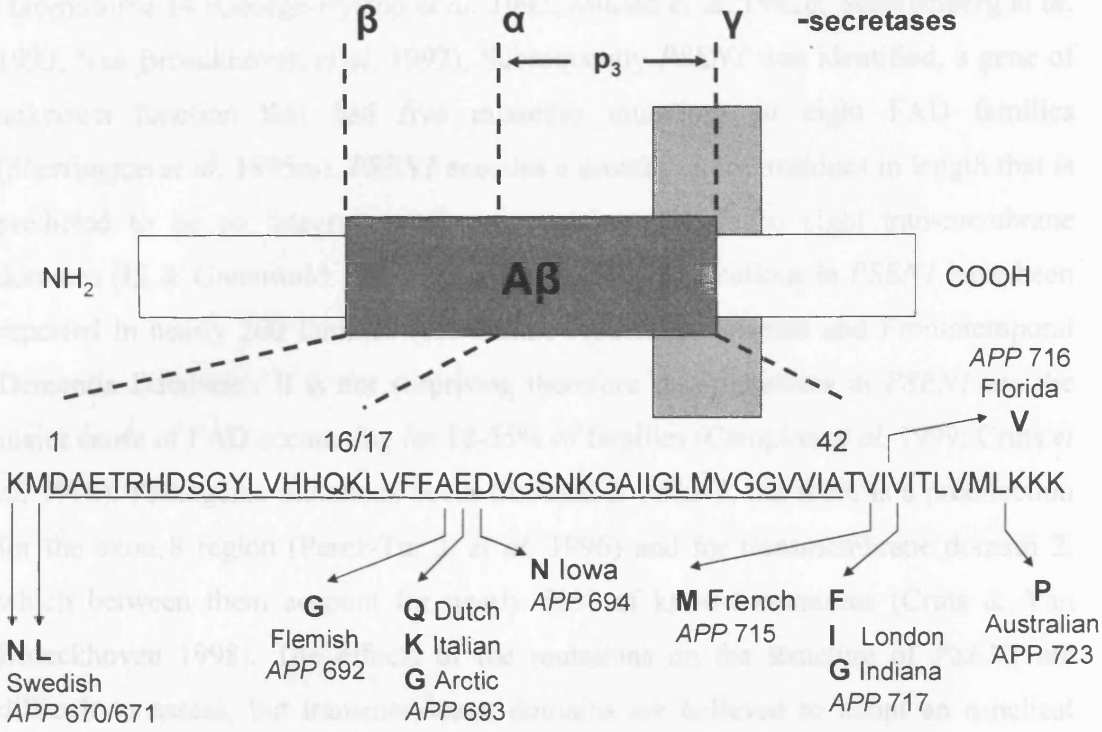
1.3.2.1 β-Amyloid Precursor Protein gene

The modern molecular era of AD research begins with Glenner and Wong's identification of the β-amyloid (Aβ) sequence in meningovascular deposits from AD and Down's syndrome brains (Glenner & Wong 1984b; Glenner & Wong 1984a). With the realisation that Aβ peptide was the subunit of the amyloid filaments in senile plaque cores (Masters *et al.* 1985) and the cloning of the β-amyloid precursor protein (*APP*) gene (Goldgaber *et al.* 1987; Kang *et al.* 1987; Robakis *et al.* 1987; Tanzi *et al.* 1987a) it would seem that it was only a matter of time before FAD mutations in *APP* were found. However, though initial linkage studies co-localised the AD gene to the same chromosomal region as *APP* (St George Hyslop *et al.* 1987; Tanzi *et al.* 1987a), the assumption that there would be a *single* AD gene led to the incorrect conclusion that this AD gene and *APP* were genetically separate (Tanzi *et al.* 1987b; Van Broeckhoven *et al.* 1987). The subsequent discovery of a missense mutation in *APP* in families with hereditary cerebral haemorrhage with amyloidosis of the Dutch type (HCHWA-D), a rare Dutch stroke disorder (Levy *et al.* 1990), showed that mutations in *APP* could lead to amyloid deposition albeit not in the brain parenchyma. This and the realisation that AD was genetically heterogeneous, led Goate *et al.* (1989) to concentrate on studies of single pedigrees. Dementia Research Group Family 23, which had previously been linked to chromosome 21, was an obvious candidate. Direct sequencing of exons 16 and 17 of *APP* (which encode the Aβ domain) revealed a point mutation at codon 717, just beyond the carboxy-terminus of the β-amyloid sequence (Goate *et al.* 1991). The mutation resulted in the substitution of valine for isoleucine (V717I) in the APP amino acid sequence which co-segregated with the affected individuals. This V717I mutation subsequently became known as the London mutation, it is the most common of the 16 *APP* mutations and has been found in 23 FAD families from the UK, France, Germany, Italy, Japan, and Thailand (see Online

Alzheimer Disease and Frontotemporal Dementia Database). The next two mutations to be identified: V717F (Murrell *et al.* 1991) and V717G (Chartier-Harlin *et al.* 1991) were both at the same residue. Residue 717 is close to the γ-secretase site and it was proposed that the effects on APP metabolism were the key to their pathogenicity. The discovery of the KM670/671NL (Swedish) mutation (Mullan *et al.* 1992a) adjacent to the β-secretase site and the A692G (Flemish) mutation (Hendriks *et al.* 1992) adjacent to the α-secretase site provided additional support for this theory. The APP mutations described to date are shown in Figure 1.1.

Mutations in APP have been shown to account for 15% of FAD families (Campion *et al.* 1999). The mechanism of action of *APP* mutations is discussed later in this thesis.

Figure 1.1 The APP molecule



The APP molecule with localization of the Aβ and p3 proteins, showing recognised pathogenic mutations.

1.3.2.2 Presenilin 1 gene

Many of the families in which EOAD had been linked to chromosome 21 did not segregate with a mutation in exons 16 and 17 of *APP*. A genome wide search was undertaken and almost simultaneously several groups reported linkage to chromosome 14 (George-Hyslop *et al.* 1992; Mullan *et al.* 1992b; Schellenberg *et al.* 1992; Van Broeckhoven *et al.* 1992). Subsequently *PSEN1* was identified, a gene of unknown function that had five missense mutations in eight FAD families (Sherrington *et al.* 1995m). *PSEN1* encodes a protein of 467 residues in length that is predicted to be an integral membrane protein with six to eight transmembrane domains (Li & Greenwald 1998). Approximately 140 mutations in *PSEN1* have been reported in nearly 200 families (see Online Alzheimer Disease and Frontotemporal Dementia Database). It is not surprising therefore that mutations in *PSEN1* are the major cause of FAD accounting for 18-55% of families (Campion *et al.* 1999; Cruts *et al.* 1998). Pathogenic mutations occur throughout *PSEN1*, but there is a predilection for the exon 8 region (Perez-Tur J. *et al.* 1996) and for transmembrane domain 2, which between them account for nearly 50% of known mutations (Cruts & Van Broeckhoven 1998). The effects of the mutations on the structure of *PSEN1* are difficult to assess, but transmembrane domains are believed to adopt an α-helical conformation, particularly transmembrane domain 2 (Clark *et al.* 1995e). Recently Hardy and Crook (2001) have mapped the known mutations onto the most widely accepted model of *PSEN1* (Li & Greenwald 1998) and have shown that many of the mutations involve the disruption of transmembrane helical faces, particularly in transmembrane domains 2, 3, 4 and 6 and possibly transmembrane domain 1. They propose that mutations cause disruption of the helical faces resulting in impaired presenilin function. The mechanism of action of *PSEN1* mutations is discussed later in this thesis.

1.3.2.3 Presenilin 2 gene

The report that demonstrated linkage to chromosome 1 in the Volga German families was "in press" at the time the article reporting the cloning of *PSEN1* was published (Levy Lahad *et al.* 1995c). As a result both groups searched for genes homologous to *PSEN1* and a second gene, which mapped into the Volga German region was identified (Levy Lahad *et al.* 1995b; Rogaev *et al.* 1995). Because of the high

homology the new gene was called *PSEN2*. The overall homology between PSEN1 and PSEN2 is 67%, with higher homology in the transmembrane domains. Mutations in *PSEN2* are a much rarer cause of FAD, having been described in only ten families including the Volga-German kindred, initially thought to be nine families until the demonstration of a founder effect (Cruts *et al.* 1998; Finckh *et al.* 2000; Lao *et al.* 1998; Levy Lahad *et al.* 1995b; Rogaev *et al.* 1995). The mechanism of action of *PSEN2* mutations is discussed later in this thesis.

1.3.3 Risk genes

1.3.3.1 The Apolipoprotein E polymorphism

For FAD due to *APP* the *APOE* ε4 allele has long been recognised as a determinant of AAO and possibly amyloid load (Alzheimer's Disease Collaborative Group 1993; Sorbi *et al.* 1995a). After initial negative reports (Levy Lahad *et al.* 1995a; Van Broeckhoven *et al.* 1994b) there have now been several studies that have shown a similar effect in *PSEN1* and *PSEN2* (Janssen *et al.* 2000; Pastor *et al.* 2003; Wijsman *et al.* 2005).

1.3.3.1 Other risk genes

Variation in AAO has also been reported both between families with the same mutation, e.g. *PSEN1* M139V (Fox *et al.* 1997) and *PSEN1* H163R (Poorkaj *et al.* 1998b), but also within families e.g. *PSEN1* H163Y (Axelman, Basun, & Lannfelt 1998). These data suggest that there are further as yet unidentified genetic factors.

A promising candidate gene is Nicastrin (*NCSTN*; OMIM 605254), a recently identified component of the presenilin containing γ-secretase complex (see later in this thesis) that is involved in the cleaving of APP and Notch in their transmembrane domains (Chen *et al.* 2001; Yu *et al.* 2000). The effect of *NCSTN* has been investigated in two population based samples of AD patients (Dermaut *et al.* 2002). These two populations were an EOAD cohort of 116 patients, of whom 78 were familial (i.e. at least one relative with dementia) with a subset of ten FAD patients (five with *PSEN1* mutations, two with *PSEN2* mutations and three without a recognised causative mutation) and 240 LOAD patients drawn from the Rotterdam study. Fourteen *NCSTN* single-nucleotide polymorphisms (SNPs) were found: 10 intronic SNPs, 3 silent mutations, and 1 non pathogenic missense mutation (N417Y).

A SNP haplotype estimation in patients with EOAD (n=116) and LOAD (n=240) indicated that the frequency of one SNP haplotype (HapB) was higher in the group with familial EOAD (7%), compared with the LOAD group (3%) and control group (3%). In patients with familial EOAD without the *APOE-ε4* allele, the HapB frequency further increased, to 14%, resulting in a fourfold increased risk (odds ratio = 4.1; 95% confidence interval 1.2-13.3; P=0.01). None of the seven FAD patients with presenilin mutations carried the HapB haplotype and it is therefore not clear whether this may be a modulator of the FAD phenotype in a different population.

1.4 Pathology of Alzheimer's disease

On macroscopic examination, an AD brain can vary in appearance from normal to severely atrophic. Typically, there is widening of the sulci and ventricular enlargement which is most marked in the lateral ventricles. There is generalised atrophy of the cerebral cortex but in addition a greater degree of atrophy of temporal lobe structures including amygdala, hippocampus and parahippocampal gyrus (Braak & Braak 1991; Gomez-Isla *et al.* 1996a). This predilection for medial temporal lobe structures can also be seen in vivo using neuroimaging, which will be discussed in detail later in this thesis. In addition to neuronal loss the definite diagnosis of AD relies on the demonstration of the histological features that were first described by Alzheimer: amyloid plaques and neurofibrillary tangles. The relative significance of these hallmark lesions is reflected in the various published criteria for the pathological diagnosis of AD, which are based on an evaluation of the frequency neuritic plaques (Mirra *et al.* 1991) or the quantitative assessment of both plaques and tangles (Khachaturian 1985). The recent consensus recommendations from the National Institute on Aging and Reagan Institute emphasized the numbers of tangles and neuropil threads in the cerebral cortex (Hyman & Trojanowski 1997).

Another major neuropathological feature of AD is cerebral amyloid angiopathy (CAA) which is characterised by amyloid deposition within the walls of arteries and arterioles within the subarachnoid space and in the cerebral and cerebellar cortex. Although CAA occurs independently, particularly in elderly individuals (Greenberg *et al.* 1993), it is present to some degree in virtually all cases of AD. Capillaries and veins may also be involved, but blood vessels in the white matter are generally spared. The amyloid tends to be associated with the vascular smooth-muscle cells or pericytes, and periarterial Aβ deposition contributes to CAA (Weller *et al.*

2000). The effect of CAA on the blood-brain barrier in AD remains controversial and is beyond the scope of this thesis, but in cases of extensive amyloid deposition within cortical blood vessels, i.e. dyshoric angiopathy, perivascular plaques maybe seen in close proximity to blood vessel walls; for a review see Kalaria (1999).

1.4.1 Pathology of Sporadic Alzheimer's disease

1.4.1.1 Amyloid plaques

Amyloid plaques are extracellular and are best visualised using Bielschowsky or Bodian silver stains or immunohistochemical techniques using an antibody to Aβ. Amyloid plaques are divided into two main types: diffuse ("pre-amyloid") plaques and neuritic plaques. Diffuse plaques are composed of homogeneous deposits of fibrillary material but contain only scant numbers of amyloid fibrils and do not stain with Congo red. Amyloid fibrils are made up of fine (7-10 nm) filaments comprised of an insoluble form of the 4 kDa Aβ peptide (Glenner & Wong 1984a; Glenner & Wong 1984b; Masters *et al.* 1985), which is derived from the larger amyloid precursor protein (APP). Neuritic plaques are more heterogeneous in composition, with a central dense core of amyloid fibrils. Dystrophic neurites occur both within this amyloid deposit and immediately surrounding it. These neurites are often dilated and tortuous and are marked by ultrastructural abnormalities including paired helical filaments, virtually indistinguishable from those that comprise neurofibrillary tangles (see below). Such plaques are also intimately associated with microglia and they are surrounded by reactive astrocytes. Neuritic plaques stain with Congo red. The key difference between the two types of plaque is that neuritic plaque Aβ occurs in the form of insoluble, and possibly neurotoxic, β-pleated sheets. Amyloid plaques are mainly observed in the neocortex, with only small numbers seen in the hippocampal formation during the early stages of AD. Plaque density in the cortex increases with disease severity. The time it takes for a neuritic plaque to develop is unknown, but these lesions probably develop over a period of many months to years.

1.4.1.2 Neurofibrillary tangles

Neurofibrillary tangles (NFTs) are intracellular structures often occupying the cell body and apical dendrite of the neuron. Neurofibrillary tangles are not specific for AD as they are found in the brains of normal aged individuals as well as in a variety of

other conditions: including progressive supranuclear palsy, dementia pugilistica, subacute slerosing panencephalitis, Niemann-Pick's disease type C, parkinsonism-dementia complex of Guam, postencephalitic parkinsonism, myotonic dystrophy, Hallervorden-Spatz disease, and Gerstmann-Straussler-Scheinker syndrome (Wisniewski *et al.* 1979). Yet, despite the fact that NFTs occur in many other conditions they remain a key diagnostic feature of AD. Although some NFTs may be apparent on haematoxolin and eosin stained sections, where they have a slightly basophilic appearance, other stains are required for optimal detection. These include a variety of silver impregnation methods, e.g. modified Bielschowsky, Gallyas, Sevier Munger and Bodian preparations. In addition, tangles are effectively demonstrated by immuno-histochemistry using antibodies directed against tau epitopes or paired helical filaments (PHFs). A monoclonal antibody, Alz50, recognizes a 68-kDa polypeptide called A68 (Wolozin *et al.* 1986). This antibody labels neurons with neurofibrillary tangles as well as many neurons that are especially vulnerable to developing tangles. This A68 protein was subsequently shown to be a major subunit of PHF, representing a phosphorylated form of tau. The general contour of neurofibrillary tangles varies with the nature of the neurons in which they reside. Tangles in the pyramidal cells of Ammon's horn in the hippocampus, for example, are usually flame shaped, whereas those in the rounder neurons of the nucleus basalis of Meynert or locus coeruleus usually have a more globose contour. Tangles in the hippocampus, particularly in CA1 and the entorhinal cortex, may survive after the neurons that harbour them have died and lost their cell membranes. These "ghost" or "extracellular" tangles, the tombstones of once viable neurons, are best detected using silver and other routine stains. Tangles in the cerebral cortex have a varied morphology depending upon the conformation of the neurons involved. Composed of two filaments measuring around 20 nm in diameter, PHFs exhibit a regular periodic constriction to about 10 nm occurring every 80 nm. Kidd's (1963) finding that this change consisted of two filaments wound in a double helix was soon confirmed (Terry 1963; Terry, Gonatas, & Weiss 1964; Wisniewski, Narang, & Terry 1976). These PHFs have since been shown to be composed primarily of hyperphosphorylated microtubule associated protein, tau (Goedert *et al.* 1992).

Braak and Braak (1991) described a hierarchical staging system for the neuropathological changes in AD, based on the distribution of NFTs in the cerebral cortex. The first neurons to exhibit NFTs and neuropil threads are the pre-α projection

neurons in the transentorhinal cortex, a transition zone between the entorhinal cortex (EC) and the adjacent isocortex. Other areas with early development of NFTs are the entorhinal cortex and field CA1 of the hippocampus. In Stages I-II (the "transentorhinal stages") these minor pathological changes are restricted to the EC and hippocampus. Stages III-IV (the "limbic stages") are characterised by moderate numbers of NFTs and neuropil threads in the transentorhinal cortex, EC and CA1, with additional scant numbers of NFTs in CA4, the subiculum and the parasubiculum. Small numbers of NFTs and neuropil threads are also found in association cortices. In stages V-VI (the "isocortical stages") all hippocampal subfields and isocortical association areas are severely affected. Primary sensory cortex is affected in stage VI.

1.4.1.3 Medial temporal lobe pathology

The vulnerability of the medial temporal lobe and specifically the EC early in the disease process is based on the observation of extensive loss of layer II neurons and widespread infiltration of stellate cells by NFTs in very mild AD (Gomez-Isla *et al.* 1996a). Tangle formation is also prominent in the pyramidal cells of layer IV, whereas layers III, V and VI are relatively free of AD pathology (Hyman, Van Hoesen, & Damasio 1990). Of the hippocampal subfields, NFT and amyloid plaque density is greatest in CA1 and the subiculum. The dentate gyrus, CA3 and CA4 contain significantly fewer NFTs and amyloid plaques, with hardly any at all in the presubiculum. There are conflicting data on the severity of neuronal loss affecting the hippocampus proper. West *et al.* (1994) reported that the most marked neuronal loss was observed in CA1, followed by CA4. The dentate granule cells and neurons in CA3 and CA2 were relatively spared. By contrast, Simic *et al.* (1997) found that there was no significant difference in neuronal loss in CA1 in AD and normal ageing, and that the greatest differences in neuronal numbers were observed in the granule cell layer and the subiculum. Neuronal numbers in CA1, CA4 and the subiculum have been found to correlate with the duration and severity of AD (Bobinski *et al.* 1998).

1.4.1.4 The pathology of normal ageing

Amyloid plaques and NFTs are also observed in the brains of nondemented aged individuals (Arriagada *et al.* 1992; Delaere *et al.* 1990). Diffuse plaques are found throughout the cerebral cortex, with additional plaques in the amygdala, EC and CA1. Smaller numbers of neuritic plaques are also found throughout the cortex, but with

relatively larger quantities in the medial temporal lobe structures (Schmitt *et al.* 2000). Neurofibrillary tangles are universally present in nondemented elderly brains (Price & Morris 1999) and are most prominent in the hippocampus and parahippocampal regions, with NFT numbers in CA1 correlating with age (Schmitt *et al.* 2000). These AD-like changes have led to the suggestion that some "normal" elderly subjects may in fact have covert, or preclinical, AD (Hulette *et al.* 1998; Price & Morris 1999). However, others argue that the different patterns of neuronal loss within the hippocampus observed in AD and normal ageing suggest that they represent separate processes (Simic *et al.* 1997; West *et al.* 1994).

1.4.2 Pathology of Familial Alzheimer's disease

It is generally agreed that FAD and sporadic AD share the same cytoskeletal pathology and are essentially indistinguishable (Lantos *et al.* 1992). More recent investigations have however, revealed that β-amyloid deposition is more severe in FAD brains. In cases due to *APP* mutations, the β-amyloid plaques are predominantly composed of $A\beta_{1\text{-}42(3)}$ with relatively little $A\beta_{1\text{-}40}$ present. The total amount of $A\beta_{1\text{-}42(3)}$ is considerably greater than in sporadic AD (Mann *et al.* 1996b). Similarly, in brains with FAD due to *PSEN1* mutations, $A\beta_{1\text{-}42(3)}$ was the main component of plaques (Mann *et al.* 1996a). Moreover, the total amount of $A\beta_{1\text{-}42(3)}$ and $A\beta_{1\text{-}40}$ was more than twice the amount deposited in cases of sporadic AD of similar duration, although the ratio between the extent of the deposition of the two amyloid species was the same compared with sporadic Alzheimer's disease. In a larger neuropathological series of AD brains from individuals with one of ten *PSEN1* or *PSEN2* mutations, enhanced deposition of total β-amyloid and $A\beta_{1\text{-}42(3)}$, although not of $A\beta_{1\text{-}40}$, in the superior temporal gyrus was noted when compared with sporadic cases (Gomez-Isla *et al.* 1999). Moreover some of the *PSEN1* mutations (M139V, I143F, G209V, R269H and E280A) were also associated with faster rates of neurofibrillary tangle formation and an accelerated neuronal loss when compared with sporadic cases of similar duration.

Some interesting neuropathological phenotypes have been reported for FAD. In the Finland A pedigree the M146V *PSEN1* mutation has been associated with mild cortical vacuolar changes (Clark *et al.* 1995e; Haltia *et al.* 1994). More prominent vacuolation and gliosis is seen in patients with the E280A *PSEN1* mutation (Lopera *et al.* 1997). Spongiform encephalopathy with "microspongiosis bubbles identical to those seen in CJD" associated with strongly positive PrP immunostaining has been

described in co-existence with characteristic AD pathology in two probands from a French family carrying the H163R *PSEN1* mutation (El Hachimi *et al.* 1996).

Pick body-like inclusions in the dentate gyrus have been reported in the AM/JPN1 pedigree which has FAD due to the A260V *PSEN1* mutation (Ikeda *et al.* 1996). In the AM/JPN1 pedigree there is a variable age at onset of 27-46 years and disease duration of 8-19 years. In addition to the neuropathological hallmarks of AD two family members who have undergone neuropathological examination had Pick-like neuronal inclusions in the dentate gyrus (silver and ubiquitin positive). This report by Ikeda *et al.* (1996) predates the recent advances in tau immunohistochemistry so it is uncertain whether these are genuine Pick bodies. This finding has not been reported in other FAD mutations and may be pathognomonic for this mutation.

Recently an atypical, yet very characteristic, clinical and neuropathological phenotype was described in a Finnish pedigree with the *PSEN1* Δ9 mutation (Crook *et al.* 1998). The affected individuals manifested progressive dementia, often preceded by spastic paraparesis. The primary and association cortices and hippocampus showed a profusion of eosinophilic, roundish structures with distinct borders termed "cotton wool" plaques (CWPs) (Brooks *et al.* 2003; Steiner *et al.* 2001; Verkkoniemi *et al.* 2000; Verkkoniemi *et al.* 2001). The CWPs were immunoreactive for $A\beta_{1\text{-}42(3)}$ but weakly or not at all for $A\beta_{40}$. They were devoid of a congophilic core, and fibrillar amyloid could not be identified within them by electron microscopy. Confocal microscopy showed reduced density of axons within individual CWPs and only few CWP related PHF tau-positive dystrophic neurites. CWPs were particularly numerous in the medial motor cortex, representing the lower extremities, and degeneration of the lateral corticospinal tracts was observed at the level of the medulla oblongata and the spinal cord. In addition to the predominant CWPs, variable numbers of diffuse and cored plaques were found in the cerebral cortex. Diffuse and non-neuritic cored amyloid plaques but no CWPs occurred in the cerebellum. Despite the significant deposition of $A\beta_{42}$, no neuritic pathology was observed in association with these unusual lesions. This together with data from cell models overexpressing the mutation suggests that amyloid plaque formation may not be a pre-requisite for dementia and neurodegeneration. This AD phenotype with spastic paraparesis associated with CWPs has now been described with several different *PSEN1* mutations and is associated with the highest Aβ concentrations described to date

(Houlden *et al.* 2000). Careful neuropathological studies of sporadic LOAD patients, has revealed a patient with numerous neocortical CWPs as well as severe CAA and marked leukoencephalopathy (Le *et al.* 2001). In their study Le *et al.* (2001) additionally studied 16 patients with LOAD with similar degrees of CAA and leukoencephalopathy. They found that CWPs were well-circumscribed amyloid deposits infiltrated by ramified microglia and surrounded by dystrophic neurites that were immunopositive for APP, but only weakly for neurofilament and PHF-tau. $A\beta_{1-12}$ was diffuse throughout the CWP, while $A\beta_{37-42}$ was peripherally located and $A\beta_{20-40}$ more centrally located. Two of the 16 LOAD patients in the comparative group also had CWPs, but they were also admixed with diffuse plaques and plaques with dense amyloid cores. Pyramidal tract degeneration was neither a consistent finding nor a prominent feature in any case. Their results suggest that CWPs are not specific for FAD with spastic paraparesis.

Considerable variation in the degree of CAA occurs in association with the presenilin mutations. In a Volga German family with N141I *PSEN2* mutation neuropathological examination revealed the presence of severe or moderately severe CAA in five family members with clinical dementia (Nochlin *et al.* 1998). The index case with the *PSEN2* mutation had late-onset dementia at age 73 years, died of an acute intracerebral haemorrhage, and pathologically showed severe amyloid angiopathy but only rare neuritic senile plaques and neurofibrillary tangles. In two Japanese families with disparate *PSEN1* mutations, a patient with the E184D mutation exhibited severe CAA whereas another individual with a N405S mutation showed only limited CAA, yet profound cortical amyloid deposition (Yasuda *et al.* 2000a). Mann *et al.* (2001) correlated amyloid angiopathy and variability in Aβ deposition in presenilin-1-linked AD to the position of the presenilin mutation. They reported a correlation between CAA and the location of mutation in FAD associated with *PSEN1* mutations. The amount of $A\beta_{42(3)}$, but not $A\beta_{40}$, deposited in the frontal cortex of the brain was increased in 54 cases of *PSEN1* FAD, encompassing 25 different mutations, compared to sporadic AD. The amount of $A\beta_{40}$ in *PSEN1* FAD varied according to the APOE ε4 dose. Although the amounts of $A\beta_{40}$ and $A\beta_{42(3)}$ deposited did not correlate with the genetic location of the mutation in a strict linear sense, the histological profile did. Patients with mutations between codon 1 and 200 showed, in frontal cortex, many diffuse plaques, few cored plaques, and mild or moderate amyloid angiopathy. Cases with mutations occurring after codon 200 also showed

many diffuse plaques, but the number and size of cored plaques were increased (even in the absence of APOE ε4) and these were often clustered around blood vessels severely affected by CAA.

Although the co-existence of Lewy body and Alzheimer lesions is recognized in sporadic AD it appears to be more common in FAD. In a series of 74 FAD cases, 22% were complicated by Lewy bodies demonstrated by α-synuclein immunostaining (Lippa *et al.* 1998). It is possible that these Lewy bodies arise from defective amyloid processing. Some cases have sufficient Lewy body pathology to consider a secondary diagnosis of dementia with Lewy bodies when applying the original Newcastle consensus criteria (McKeith *et al.* 1996). Usually however, the Lewy body pathology is minimal when compared with the overwhelming Alzheimer type lesions and there is no clinical evidence of Lewy body symptomatology to support the diagnosis.

1.5 Pathophysiology of Alzheimer's disease

1.5.1 Amyloid precursor protein structure and function

The *APP* gene encodes an alternatively spliced transcript that, in its longest isoform, codes for a single membrane spanning polypeptide of 770 amino acids (Goldgaber *et al.* 1987; Kang *et al.* 1987; Robakis *et al.* 1987; Tanzi *et al.* 1987a). Alternative splicing of exons 7 and 8 results in polypeptides of either 695 amino acids, APP_{695} expressed predominantly in brain, or of 751 amino acids (APP_{751}). The APP protein products are integral membrane proteins that contain an N-terminal signal peptide, a large ectodomain with sites for N-glycosylation, a single membrane-spanning helix and a short cytoplasmic domain (Kang *et al.* 1987).

The physiological function of APP is unknown. Knockout of the mouse *APP* gene results in fairly subtle phenotypes, including minor weight loss, decreased locomotor activity, abnormal forelimb motor activity and minor nonspecific reactive gliosis in the cortex (Zheng *et al.* 1996). This lack of a vital consequence may be explained by the fact that mammals have other genes homologous to *APP*, the amyloid precursor-like protein genes (*APLPs*) (Slunt *et al.* 1994). Cell culture studies have shown that secreted APP can function as an autocrine factor to stimulate cell proliferation and cell adhesion, and can support nerve growth factor induced neurite outgrowth (Milward *et al.* 1992; Saitoh *et al.* 1989). Other studies have indicated that APP might be able to associate with heterotrimeric GTP binding proteins to contribute

to signal transduction (Nishimoto *et al.* 1993). More recent evidence suggests two other possible roles for APP. First, it might serve as a receptor for kinesin 1 during the fast axoplasmic transport of vesicles that contain β-site APP-cleaving enzyme (BACE, see below) and presenilins; the cleavage of APP releases kinesin from the vesicular membrane which is thought to modulate the transport (Kamal *et al.* 2001). Second, the proteolytic cleavage of APP generates a labile C-terminal stub in addition to generating Aβ. This C-terminal stub is transported to the nucleus where it might function as a signal transduction molecule (Cao & Sudhof 2001), in a similar fashion to the Notch intracellular domain (Sastre *et al.* 2001).

1.5.2 Amyloid precursor protein processing

1.5.2.1 α-secretase mediated cleavage

The APP holoprotein undergoes a series of endoproteolytic events, referred to as α-, β- and γ-secretase cleavage (see Figures 1.1 and 1.2). The first of these, termed α-secretase, results from a membrane associated protease activity which cleaves APP_{695} within the Aβ domain between residues Lys_{687} and Leu_{688} (residues 16 and 17 of Aβ) resulting in two products (Weidemann, Konig, & Bunke 1989). First, a large soluble fragment the extracellular amino-terminal ectodomain of APP (APPsα) which is released into the culture medium *(in vitro)* or the cerebrospinal fluid *(in vivo)* (Palmert *et al.* 1989). Second, a membrane retained C-terminal fragment (CTF) of 83 amino acids, that is sometimes referred to as the *10-kd CTF* (Esch, Keim, & Beattie 1990; Selkoe *et al.* 1988; Weidemann, Konig, & Bunke 1989). This pathway is non-amyloidogenic, because α-secretase mediated cleavage precludes the formation of Aβ. Interestingly α-secretase does not require a strict amino acid sequence to recognise the cleavage site. Its only requirement is an α-helical domain proximal to the cleavage site, but it cleaves substrates at a well-defined distance (~16 residues) from the plasma membrane (Sahasrabudhe *et al.* 1993). It is likely that α-secretase activity is a property of several enzymes at the cell surface, including tumour necrosis factor-α (TNF-α)-converting enzyme (TACE) and ADAM17, members of the disintegrin metalloproteinase family (Buxbaum *et al.* 1998; Lammich *et al.* 1999; Parvathy *et al.* 1998).

1.5.2.2 β-secretase mediated cleavage

The formation of Aβ from APP involves two sequential cleavages, by β- and γ-secretase enzyme activities. β-secretase cleaves the extracellular domain of APP, either between Met671 and Asp672 (the +1 site), or between residues 682 and 683 (the +11 site). β-secretase cleavage results in a 99-amino-acid, membrane bound fragment (the β-stub), which is carried out by BACE1 within endocytic compartments (Sinha *et al.* 1999; Vassar *et al.* 1999; Yan *et al.* 1999). Since the homologous protein BACE2 is expressed at much lower levels in the brain it is thought to have a smaller role in APP processing in the central nervous system. The cloning of BACE and its characterisation as a standard, membrane bound aspartyl protease has resolved the identity of the molecular β-site cleavage event that generates the N-terminus of Aβ. Cleavage mediated by γ-secretase is rather more complex and is described below.

1.5.1.2 γ-secretase mediated cleavage

The β-stub is cleaved by γ-secretase leading to the formation and release of Aβ. This cleavage process involves an unusual form of proteolysis in which the β-stub is cleaved *within* the transmembrane domain at residue +40 or +42 generating $A\beta_{40}$ or $A\beta_{42}$. The Aβ peptide, particularly $A\beta_{40}$ is a normal product of cells (Haass *et al.* 1992). It is now clear that around 90% of secreted Aβ peptides are $A\beta_{40}$, a soluble form of the peptide. The remaining secreted Aβ peptides are $A\beta_{42}$ and $A\beta_{43}$ two species that are highly fibrillogenic, and are deposited selectively and early on in amyloid plaques in individuals with AD and Down's syndrome (Saido *et al.* 1995). Furthermore it is these fibrillogenic Aβ species that are secreted in vivo by FAD mutations in *APP, PSEN1* and *PSEN2* (Scheuner *et al.* 1996), providing direct evidence for the "amyloid cascade hypothesis" of Hardy and Higgins (1992). An overview of the amyloid cascade hypothesis is shown in Figure 1.2.

The nature of γ-secretase is increasingly understood and recent work on the presenilins and their associated proteins, particularly nicastrin, has resulted in the proposal that presenilin and nicastrin are essential components of a complex that confers γ-secretase activity (Yu *et al.* 2000). The details of this form of intramembranous proteolysis are emerging only now, but it also seems to be used for the processing of several other type 1 transmembrane proteins, including Notch (Chen *et al.* 2001; De Strooper *et al.* 1999); a transmembrane receptor involved in

intercellular signal transduction in embryogenesis and postnatal maturation of cells, cell proliferation and differentiation. Like *APP, PSEN1* and *PSEN2* FAD mutations (Scheuner *et al.* 1996), artificial mutations in *NCSTN* also increase $A\beta_{42(3)}$ production (Yu *et al.* 2000). Conversely cells deficient in *PSEN1* show markedly reduced production of Aβ accompanied by the intracellular accumulation of α- and β-stubs of APP (De Strooper *et al.* 1998), whereas cells deficient in both *PSEN1* and *PSEN2* show no secretion of Aβ peptides into the culture medium at all (Herreman *et al.* 2000; Zhang *et al.* 2000). The exact roles of the presenilins and nicastrin in the γ-secretase complex are unclear. Nicastrin itself is unlikely to be a protease and it has been proposed that the presenilins themselves have γ-secretase catalytic activity or alternatively that the two highly conserved aspartate residues at positions 257 and 385 might be important for the assembly of the functional γ-secretase complex (Wolfe *et al.* 1999). However, overexpression of both PSEN and NCSTN is not sufficient to generate more γ-secretase activity (Kimberly *et al.* 2002), suggesting that other factors are important. Two additional proteins APH-1 and PEN-2 have recently been shown to be physical members of the γ-secretase complex and that the combination of them with PSEN1 and NCSTN augments γ-secretase activity in mammalian cells (Kimberly *et al.* 2003). The exact mechanism of action of γ-secretase remains to be elucidated but, the contribution of APH-1 and PEN-2 to the γ-secretase complex has recently been confirmed by further studies (Prokop *et al.* 2004; Shirotani *et al.* 2004).

1.5.3 Amyloid deposition as *primum movens* in AD?

Although the influence of FAD mutations on the formation of both amyloid plaques and neurofibrillary tangles is clear, the same cannot be said for late onset AD. Though transgenic mouse models bearing FAD mutations show an increase in $A\beta_{42(3)}$ levels and subsequent plaque pathology most of these models, including bigenics (*APP* and *PSEN1* double mutants), do not show neuronal loss, tau phosphorylation or NFT formation as predicted by the amyloid cascade hypothesis (Citron *et al.* 1997; Duff *et al.* 1996; Games *et al.* 1995; Holcomb *et al.* 1998). Furthermore, in man there are areas such as the cerebellum, where there is considerable Aβ immunoreactivity, but no demonstrable neuronal loss or NFT deposition, a situation that is known to exist even in severe *PSEN1* FAD (Singleton *et al.* 2000).

This evidence gives credence to an alternative to the amyloid cascade hypothesis; this is less clearly formulated but essentially consists of a collection of

related ideas that maintain the primacy of NFT formation. This theory is further supported by the fact that tangles occur within neurons and accumulate to occupy much of the neuron and appear to result in neuronal death as signified by the "ghost tangle". Additional supportive evidence was that the progression of Aβ deposition was not thought to follow a clear hierarchical pattern nor to correlate with cognitive decline (Arriagada *et al.* 1992; Bierer *et al.* 1995; Braak & Braak 1991; Nagy *et al.* 1995). Recently, however, Naslund *et al.* (2000) reported findings from a cross-sectional post-mortem study of 79 elderly nursing home residents with clinical dementia rating scale (CDR) (Hughes *et al.* 1982) scores of 0.0 to 5.0 who died between 1986 and 1997. They compared the levels of $A\beta_{x-40}$ and $A\beta_{x-42}$ in the cortices of patients with no (CDR score, 0.0 [n = 16]), questionable (CDR score, 0.5 [n = 11]), mild (CDR score, 1.0 [n = 22]), moderate (CDR score, 2.0 [n = 15]), or severe (CDR score, 4.0 or 5.0 [n = 15]) dementia. The CDR scores were based on cognitive and functional status during the last six months of life. They found that levels of both $A\beta_{x-40}$ and $A\beta_{x-42}$ were elevated even in cases classified as having questionable dementia (CDR score = 0.5), and increases of Aβ correlated with progression of dementia. Levels of the more fibril-prone $A\beta_{x-42}$ were higher than those of $A\beta_{x-40}$ in nondemented cases and remained higher throughout progression of disease in all regions examined. Furthermore they found that increases in $A\beta_{x-40}$ and $A\beta_{x-42}$ preceded significant tau pathology in the frontal cortex, an area chosen for examination because of the absence of neuritic changes in the absence of disease. Additional evidence comes from the recent finding that a chromosome 10 locus associated with late onset AD, a risk factor for AD independent of *APOE* genotype (Myers *et al.* 2000), is also associated with increased Aβ generation (Ertekin-Taner *et al.* 2000).

1.5.4 *Tau* (MAPT) gene mutations and dementia

Paradoxically the amyloid cascade hypothesis was strengthened significantly by the discovery that mutations in *tau* (Microtubule associated protein tau - MAPT; OMIM 157140) caused a neurodegenerative dementia *per se* in the absence of amyloid deposition: frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17) (Hutton *et al.* 1998; Poorkaj *et al.* 1998a; Spillantini *et al.* 1998b). Frontotemporal dementia has a similar prevalence to AD in the presenium (Harvey, Skelton-Robinson, & Rossor 2003; Ratnavalli *et al.* 2002). It comprises a group of

disorders characterized by focal lobar atrophy, which determines the clinical features of behavioural changes and language deficits. Three prototypical clinical syndromes have been described: frontotemporal dementia - frontal variant (FTD), progressive nonfluent aphasia (PA) and semantic dementia (SD) (McKhann *et al.* 2001; Neary *et al.* 1998). The dominant features of FTD are early personality change and disordered social conduct; PA is a disorder of expressive language and SD is characterised by an impairment of semantic memory (impaired understanding of word meaning and/or object identity). In all three syndromes, perceptual and spatial functions, praxis, and memory are generally intact or relatively well preserved until the disease is advanced. Although most cases of FTD are sporadic, up to 40% of patients have a family history, a subset of whom show an autosomal dominant pattern of inheritance (Foster *et al.* 1997; McKhann *et al.* 2001; Stevens *et al.* 1998). The majority of these families have tau-positive neuronal and glial inclusions at histology, which are usually associated with mutations in *tau*. These tau-positive inclusions consist of four repeat tau. Tau is alternatively spliced in adult human brain to produce six different tau isoforms which differ in the presence or absence of exons 2, 3 and 10. Inclusion of the alternatively spliced exon 10 generates tau protein with four imperfectly repeated micro tubule-binding domains (four repeat tau) (Spillantini, van Swieten, & Goedert 2000). Mutations in *tau* lead to an overproduction of four repeat tau isoforms or to a reduced ability of tau to interact with microtubules. These mutations fall into three groups: exon 10 coding mutations, non-exon 10 coding mutations, and mutations that affect the alternative splicing of codon 10.

Over 20 *tau* mutations have been reported (see Appendix 1 for phenotypic details) and the commonest clinical phenotype is FTD. One of the more common *tau* mutations is located at the +16 position of the intron after exon 10 (OMIM 157140.0006 [MAPT, IVS10, C-U, +16]). The 10^{+16} mutation accounts for 17 families in the UK (Janssen *et al.* 2002; Pickering-Brown *et al.* 2002) out of 25 families world wide (Hutton *et al.* 1998; Poorkaj *et al.* 2001). These families have subsequently been shown to have a single common founder (Pickering-Brown *et al.* 2004). In nine DRG families with the 10^{+16} mutation the commonest presenting symptom in 23 patients was disinhibition (Janssen *et al.* 2002). A minority of family members presented with frontal dysexecutive symptoms, apathy, impairment of episodic memory or depression. All these patients subsequently developed personality and behavioural change. Memory impairment, language deficits, ritualistic behavior,

hyperphagia and hyperorality were frequent symptoms. Parkinsonism, neuroleptic sensitivity and/or primitive reflexes were present in half the patients where these data were available. Neuropathological examination of 12 patients demonstrated the hallmark tau positive neuronal and glial inclusions (Lantos *et al.* 2002) quite different from AD, though one patient fulfilled additional criteria for a definite AD diagnosis. The latter was thought to be due to an interaction of an APOE ε4 genotype. The 10^{+16} mutation destabilises a proposed stem–loop structure involved in regulating the alternative splicing of exon 10. Studies on both post mortem brain tissue and *in vitro* exon trapping experiments support the modulation of tau exon 10 splicing by the exon 10^{+16} mutation, the overproduction of exon 10 containing tau RNA isoforms and the predominant deposition of four repeat tau protein (Goedert *et al.* 1999; Hutton *et al.* 1998; Poorkaj *et al.* 2001). Ultrastructural analysis of brain tissue from patients with exon 10 splice site mutations indicates that these tau filaments appear as wide twisted ribbons, different from the paired helical filaments seen in Alzheimer's disease (Spillantini *et al.* 1997; Spillantini *et al.* 1998a).

The fact that *tau* mutations give rise to tau inclusions but not plaques and yet *APP, PSEN1* and *PSEN2* mutations give rise to both plaques and tangles suggests that amyloid pathology occurs upstream of tau pathology in AD. Additional evidence to support this comes from development of *in vivo* models of *tau* pathology subsequent to the discovery of *tau* mutations. Perhaps not surprisingly a double transgenic mouse model expressing both *APP* and *tau* mutants has both plaques and tangles (Lewis *et al.* 2001). Interestingly this bigenic mouse model has many more tangles in the limbic system and olfactory cortex than transgenic animals expressing only mutant *tau*. This suggests either that *APP* or its product Aβ influences the formation of tau tangles. Taking a different approach, Götz *et al.* (2001) directly injected $A\beta_{42}$ into the hippocampus of *tau* mutant mice and observed a dramatic increase in tangles in the amygdala, one of the regions affected in AD, allowing the same conclusion to be drawn.

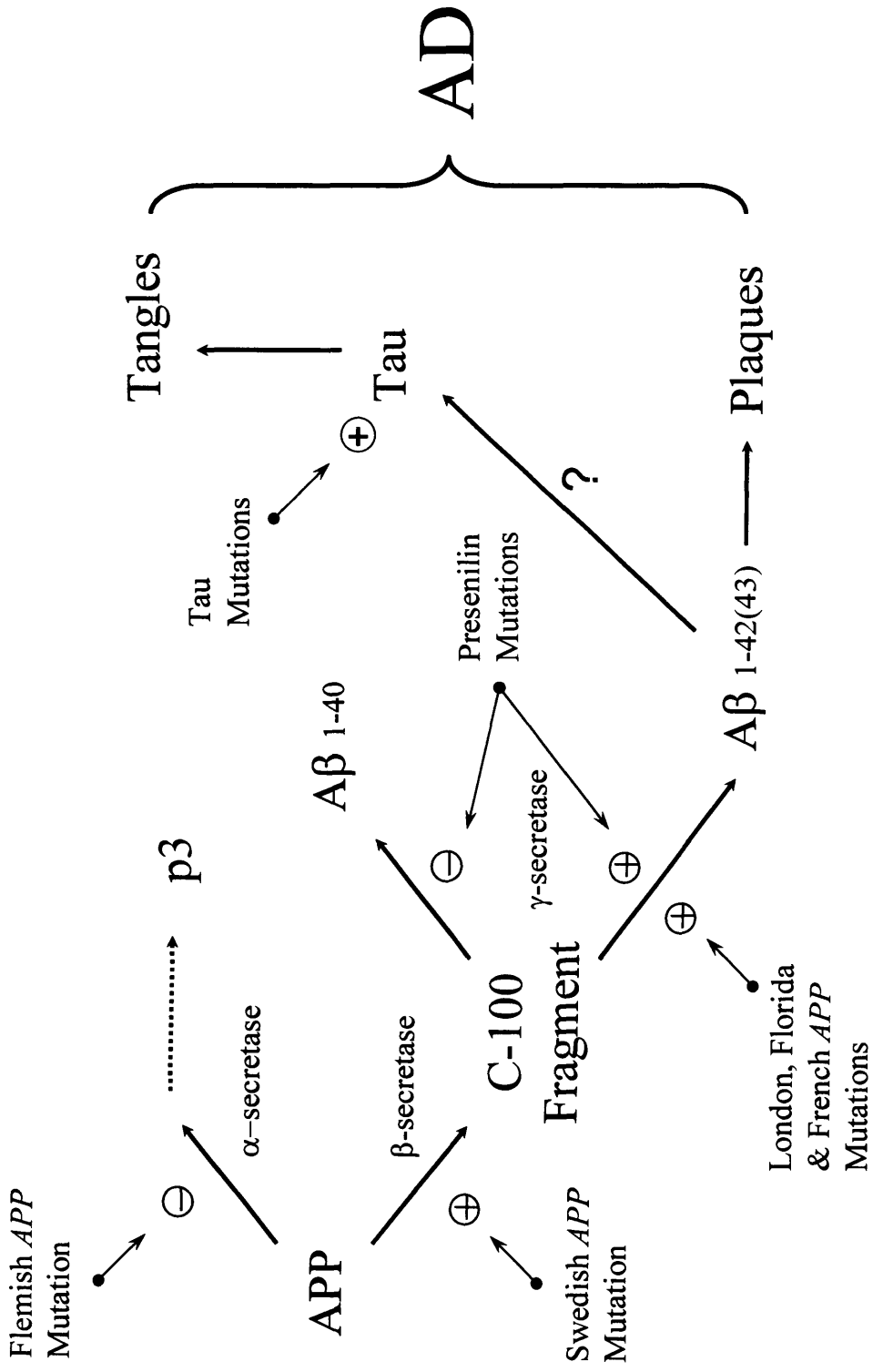
1.5.5 Immunotherapy for Alzheimer's disease

The ultimate test of the amyloid cascade theory will be whether Aβ vaccination, which both prevents and removes Aβ deposits in PDAPP mice (Schenk *et al.* 1999) and prevents their cognitive decline (Janus *et al.* 2000; Morgan *et al.* 2000), works in man. The initial excitement over these animal data has been rather tempered by the

early discontinuation of human trials after the develepmont of subacute meningoencephalitis (SAME) in a subset of patients immunized with $A\beta_{42}$ (Orgogozo *et al.* 2003). The neuropathological findings of a patient who developed SAME following vaccination with AN1792 in the Phase I study and subsequently died in February 2002 has been reported (Nicoll *et al.* 2003). In addition to diagnostic AD signature lesions there were several unusual features: (i) extensive areas of neocortex with very few Aβ plaques; (ii) those areas of cortex that were devoid of Aβ plaques contained densities of tangles, neuropil threads and cerebral amyloid angiopathy (CAA) similar to unimmunized AD, but lacked plaque-associated dystrophic neurites and astrocyte clusters; (iii) in some regions devoid of plaques, Aβ-immunoreactivity was associated with microglia; (iv) T-lymphocyte meningoencephalitis was present; and (v) cerebral white matter showed infiltration by macrophages (Nicoll *et al.* 2003). A second patient who came to autopsy following SAME showed very similar changes (Ferrer *et al.* 2004). Taken together these results suggest that an effective immune response was generated that resulted in clearance of Aβ from these patients. Moreover, it has been shown that patients who generate an antibody response exhibit slower rates of cognitive decline, an effect present even in those patients who experienced transient SAME (Hock *et al.* 2003).

It remains to be seen whether Aβ immunotherapy given early in life could prevent accumulation of Aβ and, if so, whether tau pathology might also be prevented. Studies of Aβ immunotherapy are likely to provide a crucial test of the the putative causal role of Aβ in the pathogenesis of AD.

Figure 1.2 The amyloid cascade hypothesis



1.6 Neuroimaging in Alzheimer's disease

Computed tomography (CT) and magnetic resonance imaging (MRI) are the two structural neuroimaging modalities. Structural imaging in dementia may be used to exclude structural lesions such as tumours, identify ischaemic changes and patterns of atrophy. By contrast, functional imaging can identify patterns of hypometabolism which implicate a particular disease in the setting of neurodegeneration. Functional imaging changes may exist in the absence of structural changes and may be complementary to them. The main functional imaging modalities are Positron Emission Tomography (PET), Single Photon Emission Computed Tomography (SPECT) and functional MRI (fMRI). Both PET and fMRI are largely research tools and remain confined to a small but growing number of centres. This section will only consider the relevance of structural imaging to the understanding of AD as a background to the structural imaging studies performed as part of this thesis.

1.6.1 X-ray computed tomography

CT scans are widely available, cheap and relatively rapid. A major disadvantage of CT is the radiation dose necessary for acquisition of images, but CT also has limitations in terms of image quality (DeCarli *et al.* 1990). Tissue contrast and resolution are inferior to MRI; within plane resolution is 4-5 mm when regions differ by less than five Hounsfield units in density and 1 mm when contrast between tissues is maximal. Beam hardening artefacts limit visualisation of cortical structures adjacent to bone and partial volume effects are considerable. Beam hardening effects are due to the increased absorption of lower energy X-rays by the skull which means that the medial temporal lobe is poorly visualised with conventional CT scanning. Temporal-lobe-oriented CT scanning improves visualisation and measurement of this region (Jobst *et al.* 1992). Partial volume effects refer to the mixing of signal from neighbouring tissues (e.g. CSF and brain) which results in pixels on the boundary having a value which is an average of the two adjacent tissue values. The development of spiral CT has reduced scan times and allows three dimensional reconstruction, but as yet image quality remains inferior to MRI (Bahner *et al.* 1998).

1.6.2 Magnetic resonance imaging

Structural MRI utilises the fact that hydrogen nuclei, in a magnetic field, emit a radio signal following excitation by a radiofrequency (RF) pulse at their resonant frequency. Since every cell in the brain contains water, and therefore protons, the MR dataset can give a display of the density of hydrogen nuclei (commonly referred to as proton density), slice by slice through the brain. Protons within an external magnetic field have different energy states depending on the alignment of their spin axis: parallel or anti-parallel with respect to the external field. Protons precess around magnetic field lines, the higher the field strength the higher the frequency of precession as determined by the Larmor equation (precession frequency is equal to the product of the gyro-magnetic ratio and field strength). The precession or resonance frequency is also referred to as the Larmor frequency. An RF pulse at the precession frequency of the protons has two effects on the protons: it lifts some protons to a higher level of energy (decreasing the longitudinal magnetisation) and it causes the protons to precess in phase (establishing transversal magnetisation). If after the RF pulse the longitudinal magnetisation has disappeared - resulting in only transversal magnetisation - this is termed a "90-degree" pulse. When the RF pulse is switched off the system returns (decays) to its original or natural state. The increase in longitudinal magnetisation gives rise to the T1-curve, the time constant T1 is the longitudinal or spin-lattice relaxation time. The loss of phase coherence or transversal magnetisation gives rise to the T2-curve, the time constant T2 is the transversal or spin-spin relaxation time and is much shorter than the T1 time constant.

Proton density images show little contrast between soft tissues but relatively good contrast between soft tissue and cerebrospinal fluid (CSF). Soft tissue contrast in MR is produced primarily by differences in the spin-relaxation times for protons in different types of tissue. The signal is influenced by how constrained the protons are within the particular tissue. Each type of tissue thereby produces a relatively characteristic signal with different T1 and T2 values, longitudinal spin-lattice and transversal spin-spin relaxation times, respectively. However the considerable overlap between tissues means that tissues cannot be classified solely on their proton-density, T1 and T2 parameters. The pulse sequences employed as well as the characteristics of the tissue determine the signal intensity. Images can be T1-weighted, T2-weighted, or proton-density-weighted. The degree of weighting depends on the pulse sequence,

repetition time and echo time. A short repetition time leads to T1-weighting, a long echo time leads to T2-weighting. Tissues with a large amount of freely mobile water appear dark in T1-weighted images but bright in proton density and T2-weighted images.

Variations in MR signal allow relatively sensitive differentiation of tissue types such as grey and white matter and the detection of pathological tissue or inhomogeneities within tissues. High spatial resolution (better than 1 mm within-plane) and the absence of artefact due to bone, contribute to improved tissue contrast and have allowed more accurate quantification of smaller cerebral structures. Data may also be acquired in different imaging planes and in three dimensions. There is an increasing range of different MRI procedures for acquiring images offering improved sensitivity to tissue characteristics (physical or chemical), thinner slices, or increased speed of imaging, in particular echo planar imaging (EPI) (Reiser & Faber 1997). MRI avoids X-ray irradiation, but MRI scanners are more expensive than CT and due to their physical characteristics more likely to produce claustrophobia. Longer scanning times mean MR images are sensitive to patient movement artefact which may be a particular problem in dementia where patients may forget instructions and have difficulty keeping still. Moreover, because of the powerful magnetic fields employed in MRI it is not possible to scan patients with pacemakers or ferro-magnetic clips or implants.

1.6.3 Excluding structural causes of cognitive impairment

The revised American Academy of Neurology practice parameter for the diagnosis of dementia (Knopman *et al.* 2001) recognises the inherent uncertainty of an *in vivo* clinical diagnosis of AD and recommends neuroimaging at the time of initial clinical assessment to complement the clinical assessment and to help exclude alternative pathologies which may present with a similar clinical presentation. The most important of these alternative pathologies are cerebral tumours, hydrocephalus and subdural haematomata. These are all relatively rare (Alexander *et al.* 1995), but are nevertheless the most common surgically treatable lesions that may present as dementia. Most of these pathologies will be detected by routine unenhanced CT or MR scanning. Both modalities will identify space-occupying lesions such as a frontal meningioma which may mimic frontotemporal dementia. Subdural haematomata, which can cause problems in the elderly, may also occur in the setting of degenerative

dementia. Normal pressure hydrocephalus, with its typical imaging appearances of enlarged ventricles with prominent periventricular white matter changes without corresponding sulcal widening, may produce AD-like symptoms. Normal pressure hydrocephalus can, however, be treated with ventriculo-peritoneal shunting. An unexpected scan result, for example extensive white matter changes, better seen on T2-weighted MRI, may also suggest the need for further investigation. On rare occasions this may include cerebral biopsy, to exclude a treatable condition such as cerebral vasculitis. Infectious causes of cognitive decline, particularly in the immunocompromised, may mimic a degenerative dementia and MRI and then CSF examination are essential to avoid overlooking a treatable condition.

1.6.4 Diagnostic neuroimaging in AD

The ideal diagnostic test for AD should reflect its pathophysiology, be validated neuropathologically, be able to detect early AD and differentiate it from other dementias; it should also be reliable, non-invasive and affordable (Black 1999). The NINCDS-ADRDA criteria in moderate to severely affected patients predict a neuropathological diagnosis of AD in 60-90% of patients (Davis *et al.* 1995; Risse *et al.* 1990; Tierney *et al.* 1988). It has therefore been suggested that any new diagnostic measurement must be judged by its ability to improve the certainty of a diagnosis of AD at an earlier stage of the disease (de Leon *et al.* 1993a). However, the distinction between normal ageing and mild AD using imaging is often problematic as there is overlap between these two groups due to the wide variation seen in normal controls. In order to be clinically useful any method needs to distinguish mild AD from normal ageing and ideally from other causes of cognitive impairment. Validation of a diagnostic test for AD requires comparison of an AD group with age- and sex-matched controls, where histological confirmation is available for both. Ideally the patients should be matched for pre-morbid intelligence as significant correlations between intelligence and intracranial, cerebral, temporal lobe, hippocampal and cerebellar volumes have been reported (Andreasen *et al.* 1993), although this relationship is by no means clear cut (Jenkins *et al.* 2000).

1.6.4.1 Atrophy as a diagnostic marker

Concomitant to the microscopic changes which typify the neuropathological hallmarks of AD there are macroscopic changes, such as cerebral atrophy particularly involving the temporal cortex and enlargement of CSF spaces which can be assessed with neuroimaging. Pathological studies have shown cortical atrophy, reduced brain weight and ventricular enlargement all to be more prominent in AD patients (Hyman & Trojanowski 1997; Mirra, Hart, & Terry 1993).

Whilst cortical atrophy is more prominent in AD patients there is considerable overlap with age-matched controls with a reduction in brain volume and increases in ventricular and sulcal volumes. These features are not linearly related to age, but become more important sometime after the fifth decade, although there appear to be significant variations (Coffey *et al.* 1992; Dekaban 1978; Ho *et al.* 1980). Even with correction for head size to control for variations in individual brain size there is a wide range in measures of atrophy, particularly in very elderly patients; e.g. Nagata *et al.* (1987) found a four fold range in the ratio of CSF to cranial volume in 80 year olds. Brain volume and cortical thickness gradually decline and rates of loss increase with age (Jernigan, Press, & Hesselink 1990; Pfefferbaum *et al.* 1994; Scahill *et al.* 2003).

A cross-sectional study of 194 healthy subjects ranging in age from 16 to 65 years found significant age-related increases in CSF spaces and a reduction in total brain volume of approximately 2ml per year in the 40 to 60 year age range (Blatter *et al.* 1995c). A smaller but more recent study examined 39 subjects between the ages of 31 and 84 years and found that total brain volume declined by 0.32% per year with a concomitant increase in ventricular CSF volume of 0.65mls per year (Scahill *et al.* 2003). With ageing white matter volumes appear to be relatively constant, but increasing amounts of altered signal are observed (Coffey *et al.* 1992; Erkinjuntti *et al.* 1994; Jernigan, Press, & Hesselink 1990). Inevitably there are methodological difficulties in deducing white matter and grey matter volumes using signal intensities which are themselves changing with age. Grey matter losses and ventricular enlargement increased notably after the age of 50 years (between 0.5 and 1.0 ml per year in the following decade). By contrast Jernigan *et al.* (1990) suggested there was a linear decrease with age across the whole age range, 8 to 79 years, in their measure of brain volume which they defined as "non-CSF cerebral pixels". Coffey *et al.* (1992)

studied 76 healthy adults with MRI and found a gradual decrease in overall hemispheric volume of 0.23% per year and a slightly faster decline in the volume of the frontal lobe of 0.55% per year. Temporal lobe and amygdala-hippocampal complex volumes declined at a rate that was only slightly greater than the total hemispheric rate of loss: 0.28% and 0.30% per year respectively. These age-related reductions in the hippocampus, fusiform gyrus, and parahippocampus are present even after controlling for global brain atrophy (Convit *et al.* 1995).

Studies of normal ageing are compounded by the various co-morbid pathologies that become more common with advancing age, including hypertension, atherosclerosis, diabetes and dementia. The studies described above are cross-sectional and thus open to the criticism that pre-clinical pathologies may have been present in one group, but not in another. In a longitudinal study Jack *et al.* (1998) showed hippocampal volume losses of 1.6% per year in apparently healthy elderly individuals (with a mean age of 80 years) scanned annually. There is a paucity of data on those over 85 years of age. In a longitudinal study of 46 healthy elderly individuals, including 20 subjects aged 85 years, Mueller *et al.* (1998) found rates of atrophy did not continue to accelerate with advancing age over the age of 65 years. These results are at variance with cross-sectional studies and need to be confirmed by larger studies. Confirmation of these findings would have important implications for the potential of imaging markers to differentiate AD from healthy ageing in the very elderly.

1.6.4.2 Patterns of atrophy in AD: neuropathological evidence

The histopathological changes of AD can involve nearly all cerebral areas but particularly affect the medial temporal lobe and limbic system. Braak and Braak's (1991) hierarchical staging system, based on the distribution of NFTs in the cerebral cortex, shows early NFT accumulation in the entorhinal cortex and hippocampus. These findings are in accord with the clinical observation that memory impairment is one of the earliest manifestations of AD (Hodges & Patterson 1995; Newman *et al.* 1994). The causal links and time course of this process are not established, but these neuropathological changes are known to be associated with atrophy. This atrophy can be quantified with imaging and provides an indirect method of *in vivo* assessment of the underlying disease process. Imaging studies have tended to assess global cerebral

atrophy or have focused on region specific atrophy, particularly hippocampal and amygdala atrophy.

1.6.5 Qualitative assessments of atrophy

Present clinical practice in a case of probable AD, consists of excluding causes such as space occupying lesions and then making a judgement of the amount of cerebral atrophy and the extent of white matter change. Qualitative ratings of global atrophy, when formalised, use a scoring system for the size of specific structures or of ventricles and/or sulci as markers of atrophy.

The sensitivity of qualitative assessments of generalised cerebral atrophy on CT are limited to no better than 60%, with a specificity of 90%. In a comprehensive review DeCarli *et al.* (1990) concluded that the sensitivity of this method was limited to slightly more than a chance level of 50%. Qualitative assessments of temporal lobe atrophy (temporal horn enlargement, reductions in the size of the hippocampus, medial and lateral temporal cortex) result in improved sensitivity and specificity, although 20% or more of normal controls or AD patients are still incorrectly classified (George *et al.* 1990; Kido *et al.* 1989; LeMay *et al.* 1986).

To address these shortcomings of CT, The Consortium to Establish a Registry for Alzheimer's Disease (CERAD) assessed the reliability of standardised (axial T2-weighted spin echo and T1-weighted coronal sequences) qualitative MRI evaluation for AD. This multi-centre study found that inter-rater agreement of the MRI scans was unsatisfactory and concluded that "eyeball" interpretation of MRI scans was highly subjective and that more objective techniques were required (Davis *et al.* 1992).

Since then several qualitative rating scales have been developed to assess temporal lobe atrophy on MRI. Studies have continued to show an overlap between the patient and control groups, particularly when mildly affected subjects were studied. For a specificity of 90% the sensitivity of ratings of atrophy have varied from 40% to 95%, the latter using entorhinal cortex (Davis *et al.* 1992; de Leon *et al.* 1997; Erkinjuntti *et al.* 1993; Horn *et al.* 1996; Scheltens *et al.* 1992b). Visual assessment of dilatation of the perihippocampal fissures has been shown to be predictive of cognitive decline in minimally impaired individuals with a sensitivity of 91% and a specificity of 89% (Convit *et al.* 1993; de Leon *et al.* 1993b). The same group found hippocampal atrophy in 96% of severely affected AD patients but also in 48% of subjects aged 76-90 years of age (de Leon *et al.* 1997). Scheltens *et al.* (1997) have

extensively investigated the use of visual assessment of cerebral and medial temporal lobe atrophy in the assessment of AD. When atrophy ratings on a 0-3 scale of 13 regions were performed independently by four raters relatively poor inter-rater agreement was found. They concluded that this assessment of cerebral atrophy on MRI in an elderly population had poor reproducibility among raters. However, it provided regional atrophy measures and the authors felt the method was quite reliable when performed by a single rater. Assessment of medial temporal lobe atrophy appears more sensitive than assessment of generalised atrophy. However, while the presence of atrophy on visual inspection makes the diagnosis of AD more likely, the absence of atrophy does not exclude the diagnosis.

1.6.6 Quantitative measures of atrophy

In order to achieve greater objectivity several quantitative measurements of atrophy have been developed. These measurements may be linear, of cross-sectional area or of volume. To correct for pre-morbid normal variation in brain size these measurements should be normalised (Bergin *et al.* 1994; Free *et al.* 1995; Jack *et al.* 1997; Pfefferbaum *et al.* 1994). The most commonly used denominators are total intracranial width, area or volume. Some studies have compared volumes of a region of interest with that of another structure deemed to be relatively unaffected by the disease, such as the lenticular nucleus (Seab *et al.* 1988).

1.6.6.1 Linear measurements

Linear measurements are generally simple and quickly performed as they consist of the measurement of the distance between two recognisable anatomical landmarks. Using this method the widths of the lateral ventricles and the third ventricle in several CT studies have been shown to be significantly different between AD and normal controls, although the sensitivity was no better than 70% (DeCarli *et al.* 1990). Using a single linear measurement of the minimum width of the medial temporal lobe on temporal-lobe-oriented CT, Jobst *et al.* (1992) were able to reliably differentiate AD patients (severely affected with a mean MMSE of 9/30) from normal controls. Using this technique the same group went on to demonstrate a greater rate of reduction in the minimum width of the medial temporal lobe in patients with AD compared with controls when these were followed longitudinally (Jobst *et al.* 1994). Linear measures of ventricular size on MRI have been shown to correlate with dementia severity

(Schmidt 1992). Although the maximal transverse width of the temporal horns on MRI differs significantly between mild AD and controls there is considerable overlap (Erkinjuntti *et al.* 1993).

1.6.6.2 Area measurements

Ventricular size has been estimated by measuring the cross-sectional area of the ventricles divided by total brain area at a single defined level on CT or MRI. This measurement, termed ventricular brain ratio (VBR), has been shown to increase with age in normal controls. Patients with AD have significantly increased areas of the third ventricle, lateral ventricles and interhemispheric fissure when compared with normal controls. These measures have been used with CT to distinguish AD patients from controls, and though more sensitive than linear measures they have not been shown to be clinically useful in early AD or in the differential diagnosis of dementia (DeCarli *et al.* 1990).

Using MRI, Seab *et al.* (1988) measured hippocampal cross-sectional area at the level of the lateral geniculate body and found a 40% reduction in mean normalised hippocampal area in the AD patients compared with controls. This was a small study with only ten patients and seven controls but importantly there was no overlap between patients and controls. Interestingly there was no correlation between the hippocampal measurements and disease severity. Similar changes were shown by Ikeda *et al.* (1994) using single normalised area measurements of the hippocampal formation, the parahippocampal gyrus and the temporal lobe in patients with possible (n=6) and probable AD (n=8) (MMSE 21-27/30) and in controls (n=8). Both the normalised hippocampal formation and parahippocampal gyrus areas were significantly smaller in the AD groups than in the controls but with some overlap. Although the temporal lobe measurements were only smaller in the probable AD group they failed to differentiate between patients with possible and probable AD. They concluded that hippocampal and parahippocampal atrophy occurs early in AD, and is more useful than neocortical atrophy for early diagnosis. Conversely Cuenod *et al.* (1993) demonstrated that the hippocampus measured from a single slice did not differ significantly between mild AD and normal controls.

Although area measurements may provide a better estimate of atrophy than linear measures they may not accurately estimate volume change. This is because a single slice has to be chosen for measurement and the volume change may not be

uniform along the length of the cerebral structure being measured (Cook *et al.* 1992). Furthermore because of normal anatomical variation between subjects it is difficult to ensure that the equivalent section of a structure is being measured.

1.6.6.3 Volumetric measurements

Cerebral structure volumes are estimated from image slices using Cavalieri's principle: a region or area of interest is defined on a number of slices and then the area measurements are summed and multiplied by the distance between the cross-sections measured (Free *et al.* 1995). This is identical to voxel counting methods based on slice-by slice region delineation. The smaller the distance between slices measured the greater the accuracy of the estimate, until the distance becomes of the same order as the within-plane resolution. The methods used to define the structure seen on the slice vary from manual outlining using anatomical landmarks to an automated segmentation. Automated methods use thresholds, either Hounsfield units (CT) or grey scale level (MRI), to distinguish CSF, grey and white matter on the basis of signal intensity. Though MR imaging produces greater distinction between tissue types, calculated volumes are influenced by the intensity level that is set for each image (Harris *et al.* 1994). Partial volume averaging, i.e. the mixing of signal from adjacent regions, limits accuracy but may be partly compensated for when two or more MR sequences are combined (Jack 1991; Rusinek *et al.* 1991).

In the largest study to have compared area and volume measurements Laakso *et al.* (1998) showed hippocampal volumetry to have significantly better discriminative power than area measurements. They compared these measures in 160 subjects and found the discrimination based on volumetry resulted in an overall correct classification of 92% of AD patients versus non-demented elderly subjects, whereas discrimination based on hippocampal area was less accurate, producing a correct classification in 80% of the subjects.

1.6.6.4 CSF volumes

Early CT derived measurements showed that mean CSF volume increased with severity of AD with significant differences in CSF volume between AD patients and normal controls (sensitivity and specificity approaching 90%) (DeCarli *et al.* 1990; Ichimiya *et al.* 1986). The sylvian fissure and temporal horn CSF volumes appear to

have the greatest discriminatory value (Sandor *et al.* 1992; Sullivan *et al.* 1993). Longitudinal studies suggest that cognitive performance correlates with rates of change in ventricular CSF volumes (Luxenberg *et al.* 1987). Sulcal or extraventricular CSF volumes are difficult to estimate using CT due to beam hardening artefact from adjacent bone.

MRI allows improved separation of all CSF spaces and validation studies suggest reasonably high accuracy and low interobserver variability (Jack 1991). MRI with its higher definition of extraventricular CSF has shown the increase in CSF spaces to include, to a lesser extent, sulcal CSF (Tanna *et al.* 1991). When comparable CSF regions in the same subjects are assessed, MRI is better than CT at separating AD from controls (Sandor *et al.* 1992).

1.6.6.5 Grey and white matter volumes

Concomitant with increasing ventricular and sulcal spaces, total MRI brain volume measurements are reduced in AD compared with controls. Tanna *et al.* (1991) used T2-weighted MRI to show that mild to moderately affected AD patients have 7% less total brain volume than controls. Pantel *et al.* (1997) found that in moderately affected patients (mean MMSE = 17) brain volumes were reduced by 12% relative to controls, when corrected for intracranial volume. However, their AD patient group was almost ten years older than the controls and so some of this difference may be age-related.

Rusinek *et al.* (1991) used two MR inversion recovery sequences to compensate for partial signal averaging and assign voxels to either grey or white matter compartments or CSF and found that the percentage of brain tissue identified as grey matter in AD patients was significantly lower than in control subjects, 45% as opposed to 50%. Loss of grey matter is strongly associated with increases in CSF in each cortical lobe. Automated tissue segmentation techniques have given consistent results regarding the contribution of white matter losses to cerebral atrophy. Tanabe *et al.* (1997) suggested that in mildly affected individuals white matter volumes were unchanged. However, such conclusions must be treated cautiously because automated tissue segmentation routines require the signal characteristics of brain tissues to be relatively unaffected by the disease. It is likely that both grey and white matter losses contribute to reduced brain volume even in early disease (de la Monte 1989; Matsumae *et al.* 1996; Rusinek *et al.* 1991).

Overall lower brain volumes correlate with gross measures of severity of cognitive impairment. However, as might be expected, the uncorrected brain volume measurement has little diagnostic value due to the wide range in normal brain size (Murphy *et al.* 1993). The brain normally occupies 85% to 92% of the intracranial volume and consequently relatively small decrements in brain size lead to large increments in ventricular and/or extraventricular volumes (Matsumae *et al.* 1996). This might imply that the CSF measures would be sensitive markers of disease, however, in addition to large normal variations in CSF volumes the coefficient of variation in CSF measurements has previously been unsatisfactory. Very considerable overlap is present when mildly or even moderately affected patients are studied. Tanabe *et al.* (1997) concluded from their MRI study of mild AD that "tissue segmentation may increase our understanding of dementia but as yet, when used alone, it does not play a role in the premorbid diagnosis".

1.6.7 Regional volumetric measures

The prominent neuropathological involvement of the medial temporal lobes and the hippocampal formation in AD has resulted in the atrophy of these structures being extensively studied and there is now a considerable imaging literature which is reviewed briefly in this section.

1.6.7.1 Medial temporal lobe atrophy

Several studies have shown that temporal lobe volume reductions in AD are proportionately greater than whole brain volume losses (Jernigan *et al.* 1991; Rusinek *et al.* 1991). DeCarli *et al.* (1995) measured the volumes of several brain structures and CSF spaces in 19 men with AD (mean MMSE of 16) and 18 age-matched controls. The AD group had significantly smaller mean cerebral and temporal lobe volumes, and significantly larger mean ventricular and temporal lobe peripheral CSF volumes than did controls. Mean volume of temporal lobe brain matter was found to have decreased significantly more than whole brain volume. Thirteen studies involving over 400 patients with mildmoderate AD were recently reviewed: hippocampal and medial temporal structure volumes detect dementia from normal ageing with an average sensitivity of 85% and specificity 88% (Bosscher & Scheltens 2002).

1.6.7.2 Hippocampal atrophy

Of the temporal lobe structures the hippocampus is relatively easy to separate from surrounding tissue by manual outlining and has consistently been shown to have volume loss in severe AD. In an early study Kesslak *et al.* (1991) reported a 40% reduction in the hippocampus and the parahippocampal gyrus using only five coronal slices to estimate volumes. Jack *et al.* (1992) with a larger sample (20 patients and 22 controls) showed that normalised volumes of anterior temporal lobe and hippocampal formation were both significantly smaller in the AD patients. However there was some overlap between patients and controls; the hippocampal formation was the best discriminator with 85% (17/20) of patients falling outside the range for the controls. Pearlson *et al.* (1992) measured the amygdala as well as the hippocampus by area measurements on only two MRI slices. Using this method they compared 15 moderately severe AD patients with 16 normal controls and found that their AD patients had a 30% reduction in each of these structures. A similar volume reduction (33%) in the amygdala of AD patients compared with controls was found by Cuenod *et al.* (1993), though they showed a less pronounced and non-significant reduction in hippocampal volume.

Studies of patients described as mildly affected (MMSE > 21) have shown that on average the hippocampal formation has already lost 25% or more of its volume when compared with normal controls (Killiany *et al.* 1993; Krasuski *et al.* 1998; Laakso *et al.* 1995; Lehericy *et al.* 1994). In a large and carefully conducted study Jack *et al.* (1997) used MR-based volumetry to measure the hippocampus, parahippocampal gyrus, and amygdala in 94 individuals with probable AD and 126 controls. Volumes of each structure declined with increasing age in control subjects. The volume of each medial temporal lobe structure was significantly smaller in AD patients than control subjects. The hippocampal measurements were best at discriminating control subjects from AD patients. Hippocampal volumes declined with disease severity.

Hippocampal volumetry using MRI has been used in an attempt to distinguish patients with MCI from normal ageing. Cross-sectional studies have suggested that measures of hippocampus or entorhinal cortex may be useful predictors as to which patients will proceed to develop AD from either MCI or normal ageing (De Santi *et al.* 2001; Dickerson *et al.* 2001; Du *et al.* 2001; Krasuski *et al.* 1998; Rusinek *et al.*

2003). Furthermore studies of patients destined to get AD on the basis of a mutation in *PSEN1* or *APP* have been shown to have reduction of medial temporal lobe volumes prior to the onset of symptoms (Fox *et al.* 1996; Schott *et al.* 2003).

1.6.7.3 Entorhinal cortex

Braak and Braak's (1991) neuropathological staging studies have resulted in several groups looking at MR volumes of the entorhinal cortex (EC). Juottonen *et al.* (1998) measured EC in 30 AD subjects (mean MMSE = 21) and 32 controls and found that normalised volumes were reduced by almost 40%. In a later study using the same subjects they showed that both EC and hippocampal volumes classified control subjects and Alzheimer's disease patients with a high degree of accuracy (approximately 90%) with hippocampus performing marginally better (Juottonen *et al.* 1999). De Leon *et al.* (2001) outlined what they termed the entorhinal landmark surface area on thin slice T1-weighted MRI and then corrected for intracranial volume. This area measurement was 27% lower in eight patients with very mild AD than in eight matched control subjects while in the same patients the hippocampal volume was only reduced by 12%. Killiany *et al.* (2002) compared MRI volumes of the entorhinal cortex and the hippocampus in normal controls (n=28), MCI patients who did not progress (n=73), MCI patients who progressed to AD (n=21), and patients with mild AD at baseline (n=16). They found that measures of both the EC and the hippocampus were different for each of the pairwise comparisons between the groups. However, the volume of the EC differentiated the subjects from those destined to develop dementia with considerable accuracy (84%), whereas the measure of the hippocampus did not. The findings in MCI and early AD that the atrophy rate in the EC is higher than in the hippocampus is consistent with the view that AD pathology begins in the entorhinal cortex. These studies suggest that entorhinal cortex measurements would be powerful aids to the early diagnosis of AD.

1.6.8 Longitudinal studies

Cross-sectional studies have the disadvantage that small changes in brain structure tend to be masked by the large variation within normal individuals. Hence scans of affected patients may be reported as normal as they lie within the normal range for the population as a whole. Longitudinal studies have the advantage of being able to use each individual as their own control but necessitate serial scans. Longitudinal studies

also allow the assessment of early change and the correlation of symptom onset with structural change. Even with longitudinal studies, detection of small, diffuse change remains difficult. Manual volumetric measurement involves outlining particular regions of interest and there is a critical dependence on the reproducibility of identifying anatomical structures and on *a priori* decisions about which areas to measure. Furthermore, variations in scan acquisition can make significant differences to the rates of change measured; In addition there are inevitable changes in the MRI magnetic field that occur over time due to scanner gradient changes. These changes can result in fluctuation in voxel size, further complicating comparison (Gunter *et al.* 2003; Whitwell *et al.* 2001).

Many of these criticisms are addressed by using the method of rigid body serial registration of MRI scans, in which patients act as their own controls (For review see Ashburner (2003)). Several automated techniques have been designed to allow accurate scan matching (Freeborough, Woods, & Fox 1996; Woods *et al.* 1998). These algorithms compare signal intensities from all voxels within the brain. The aim is to superimpose voxels of a similar signal intensity (i.e. grey voxels to grey voxels; white voxels to white voxels), thereby using all the information within the scan to perform the matching process. Freeborough *et al.* (1996) described such a voxel intensity based method of registration, employing an optimisation procedure based on minimising the standard deviation of voxel intensity ratios. The basic registration involves: (1) segmenting (scalping) the scan to remove non-brain tissues (e.g. skull and scalp); (2) determining and applying a series of rotations and translations to match a repeat scan to the baseline. Three translations are applied in each direction within the x, y, and z axis and three rotations around these axes produce a so-called *six degrees of freedomregistration – 6dof.* The process can be extended to allow voxels to stretch to a small extent in each of the three planes (*nine degrees of freedom registration – 9dof*). This theoretically allows for correction of inconsistencies in voxel size that may occur between the two scans. Once registered, a difference image can be created, so that change between scans can be visualized (see Figure 1.3). This validated technique permits accurate study of changes over time and the quantification of such changes using the brain boundary shift integral (BBSI) (Fox, Freeborough, & Rossor 1996). In essence the BBSI calculates the shift at the brain/CSF boundary at every point across the three dimensional registered scan-pair, the sum of which approximates closely to the brain volume lost; being a direct

measure this leads to a reduction in the error associated with indirectly comparing two scans (Freeborough and Fox, 1997). This process is shown diagrammatically in Figure 1.4.

Non-linear registration allows for a more accurate matching of gyral anatomy by allowing scans not merely to match through movement as a rigid body, but additionally through a warping procedure. One such approach is fluid registration (Christensen *et al.* 1996; Freeborough and Fox, 1998), which models the transformation from one scan to another based on the physical properties of a viscous compressible fluid. Following a 9dof rigid body registration the model aims to find an exact match between source and target scan; in so doing a displacement vector is generated for each voxel within the image, with the degree of stretch or contraction demonstrating atrophy or expansion over the period; this can be demonstrated as an overlay image (voxel compression map - VCM), providing an unbiased means of demonstrating the pattern of regional atrophy occurring between two scans without the need for a priori decisions as to which structures should be assessed. This technique allows an individual's pattern of progressive atrophy to be visualized. Fluid registration has also been shown in pilot studies to be useful in propagating outlined hippocampal volumes forward from a baseline scan to a registered repeat, thus potentially increasing accuracy and reducing time in making hippocampal measurements (Crum *et al.* 2001); however at present fluid registration is generally used to demonstrate qualitative rather than quantitative patterns of atrophy. Other computational methods for determining atrophy patterns in an unbiased fashion have also been developed and are summarized by Ashburner *et al.* (2003).

1.6.8.1 Global brain atrophy

Global brain volume loss can be assessed using manual measures of whole brain or CSF spaces or automated techniques (e.g. the BBSI). Ventricular expansion is an inevitable consequence of atrophy of brain tissue, and can be reliably and accurately measured from serial scans. Studies suggest that ventricular expansion occurs at approximately 5-8ml/yr (DeCarli *et al.* 1992; Luxenberg *et al.* 1987; Shear *et al.* 1995; Silbert *et al.* 2003; Wang *et al.* 2002), far higher than the 0.7-1ml per year seen in healthy controls (Scahill *et al.* 2003; Schott *et al.* 2003; Wang *et al.* 2002). Measures assessing whole brain change using several methods including the direct automated BBSI technique have determined rates of whole brain atrophy in AD to be

approximately 2-2.4%/yr far in excess of those reported in normal ageing (Bradley *et al.* 2002; Fox *et al.* 1999; Fox *et al.* 2000; O'Brien *et al.* 2001; Wang *et al.* 2002). Furthermore brain volume loss correlates well with dementia severity (Fox *et al.* 1999). Brain volume loss and ventricular enlargement are highly correlated, but this relationship may theoretically be altered by treatments or by pathology (e.g. obstructive hydrocephalus).

1.6.8.2 Atrophy of temporal lobe structures

Rates of hippocampal and medial temporal lobe atrophy have all been shown to be significantly greater in AD than in normal controls when subjects are rescanned after an interval of a year or more both in CT (de Leon *et al.* 1989; Jobst *et al.* 1994) and MRI studies (Jack *et al.* 1998; Jack *et al.* 2000; Jack, Jr. *et al.* 2003; Kaye *et al.* 1997; Laakso *et al.* 2000). Some of these studies are summarised in Table 1.2. There is excess atrophy compared to controls in all studies. For hippocampal atrophy there is large variability between studies, and in some cases there is also significant within-study variation (reflected by a large standard deviation). The former is likely to be due to a combination of measurement error and differences in measurement protocols; the latter to be due to inter- and intra-rater variability in measuring the hippocampus, as well as the possibility that the hippocampus might undergo different rates of atrophy at different disease stages. Studies in FAD have demonstrated that excess hippocampal and entorhinal cortex atrophy is probably occurring years prior to the clinical manifestation of the disease (Fox *et al.* 1996; Schott *et al.* 2003), and excess medial temporal lobe atrophy can predict which patients will proceed from a diagnosis of MCI to AD (Jack *et al.* 2000). Conversely it may be that the rate of medial temporal atrophy decreases later in the disease process (Scahill *et al.* 2002; Silbert *et al.* 2003), perhaps when the hippocampus has little volume left to lose.

Although entorhinal cortex atrophy has been reported to undergo both earlier and higher rates of atrophy than the hippocampus, most authors conclude that hippocampal measures (which are less prone to measurement error) are likely to be overall a more practical marker of disease progression (Jack, Jr. *et al.* 2004; Schott *et al.* 2003; Xu *et al.* 2000).

1.6.8.3 Conclusions

Early medial temporal lobe atrophy, accompanied by increased rates of whole brain atrophy occurs in AD. The rate of MTL atrophy is likely to be higher than that of whole brain atrophy particularly in early AD, but is likely to show more variability due to measurement errors associated with manual outlining, and possibly variability in rate over the course of the disease. Rates of either medial temporal lobe or whole brain atrophy are increased in AD compared to age-matched controls.

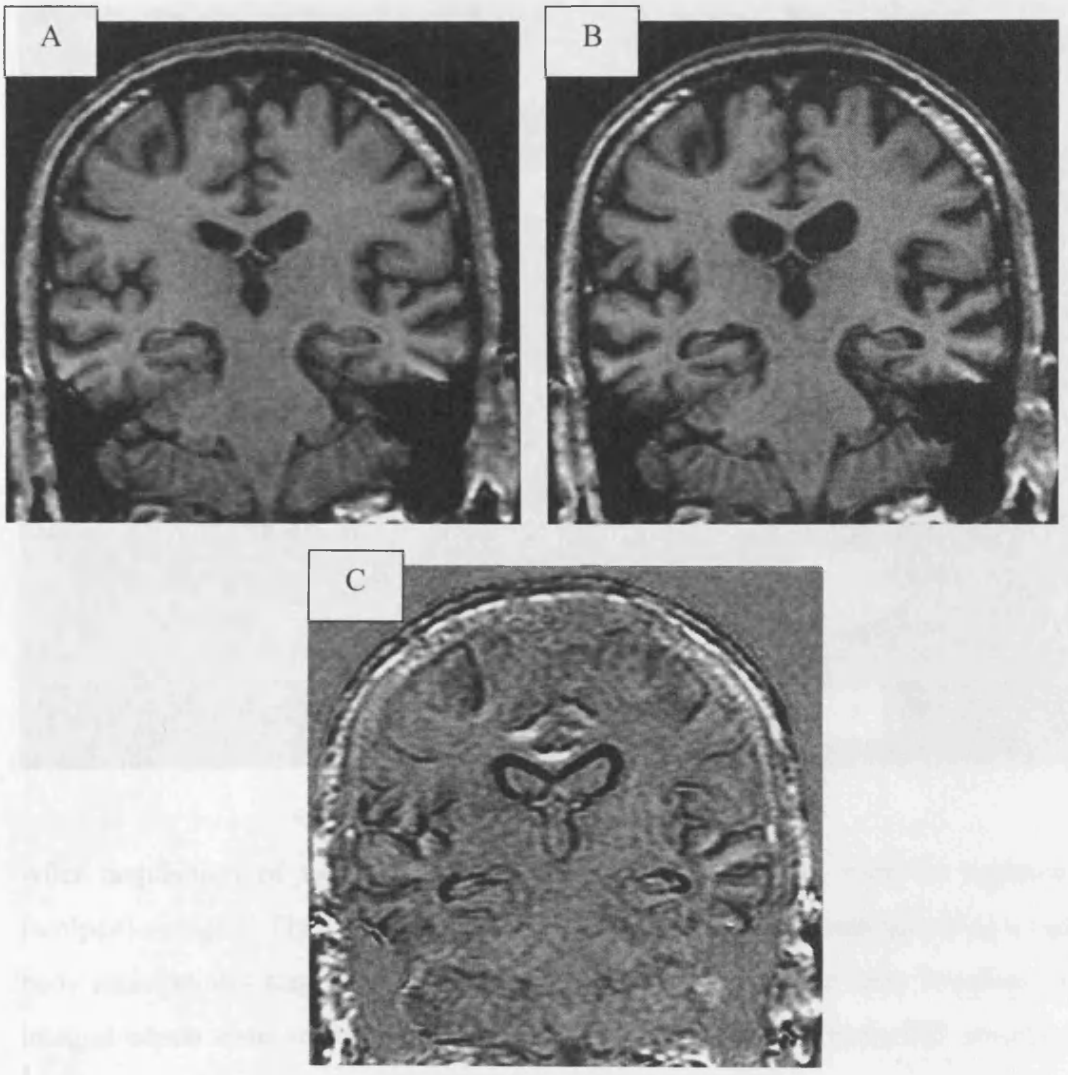
1.6.9 Detection of early change

By definition retrospective studies of AD patients cannot detect presymptomatic changes in AD. In order to fulfil criteria for a diagnosis of probable AD progressive multiple cognitive deficits must be present. In order to detect the very earliest changes of AD asymptomatic subjects need to be studied prospectively. The incidence of AD for unselected healthy individuals from the general population is actually quite low, and a prospective imaging study would require a very large number of subjects to be studied in order for the sample to include sufficient numbers of subjects who will become affected during the study. Enriching the study population by selecting subjects at *high(er) risk* of AD would make a prospective imaging study more feasible. The most robust risk factors for AD include: age, family history, and *APOE ε4* homozygosity. Suitable alternatives would be the recruitment of the very elderly (85 years of age and over), those with cognitive impairment but not (yet) fulfilling criteria for AD e.g. those with MCI or asymptomatic individuals from FAD pedigrees entering the age range of highest risk for symptom onset. The FAD group avoids the problems associated with co-morbid pathologies (e.g. vascular disease) and reduces the risk of censuring of the cohort by unrelated pathologies. Furthermore in the FAD group the development of cognitive decline can almost certainly be ascribed to AD even without neuropathological confirmation.

Table 1.2 Rates of Change in Alzheimer's Disease

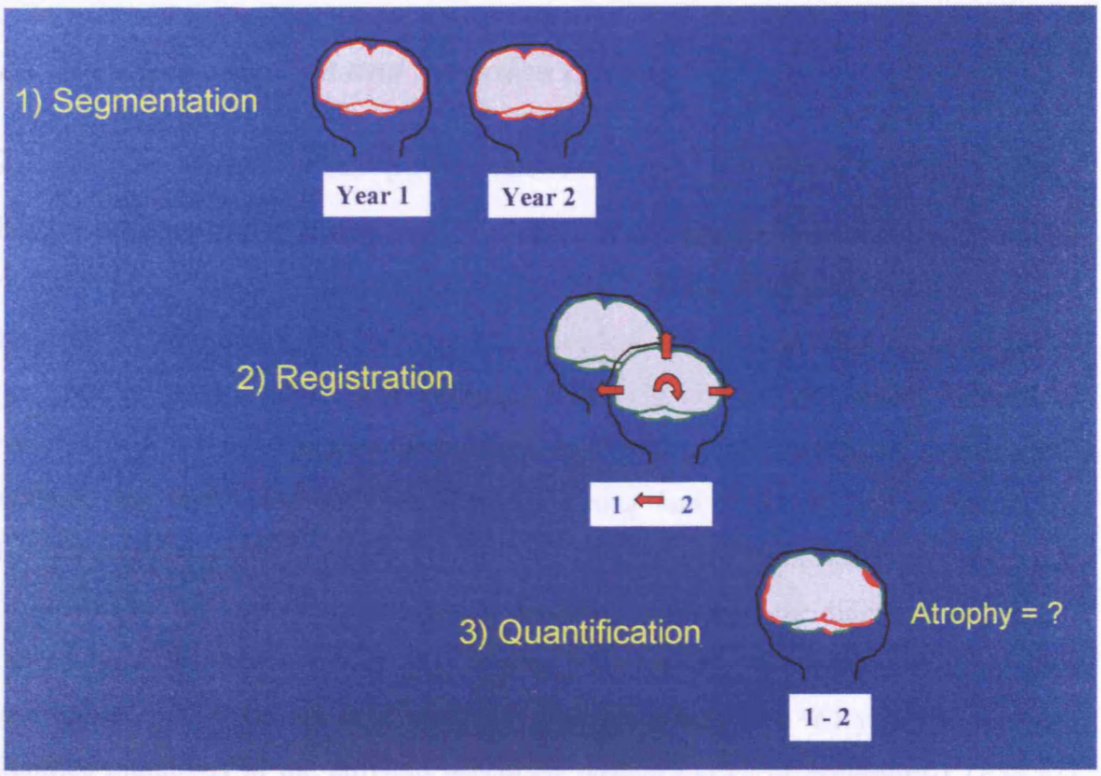
Author CT/MRI	Structure	AD Mean (±SD) loss per year	Control Mean (±SD) loss per year
Luxenberg (1987) CT	Lateral ventricles	-13 (8)cc	-0.5 (1.2)cc
Burns (1991) CT	Vent-brain ratio Third ventricle Total cortex	9.3 (12.6) % -16.6 (33.3) % 6.8 (13.6) %	-/-
DeCarli (1992) CT	Lateral ventricles	-7.3 (9)cc	-0.6 (2)cc
Jobst (1994) CT	Minimum thickness of medial temporal lobe	15.1 (11.6)%	1.5 (4.5)%
Shear (1995) CT	Ventricular system	-5.28 (5.89)cc	-0.61 (2.55)cc
Kaye (1997) MRI	Hippocampus Parahippocampus Temporal lobe	2.3 (2.0) % 2.9 (2.3) % 1.24 (1.41) %	2.1 (3.5) % 2.2 (2.4) % 0.01 (1.67) %
Fox (1997) MRI	Brain	2.78 (0.92)%	0.24 (0.32)%
Jack (1998) MRI	Hippocampus Temporal horn	3.98 (1.92) % -14.16 (8.47)%	1.55 (1.38) % -6.15 (7.69) %
Jack (2000) MRI	Hippocampus	3.5 (1.8) %	1.7 (0.9) %
Fox (2000) MRI	Brain	2.37 (1.11)%	0.41 (0.47)%
Laakso (2000) MRI	Right hippocampus Left hippocampus	6.9 (18.2) % 7.2 (20.1) %	1.6 (15.1) % 3.6 (15.1) %
O'Brien (2001) MRI	Brain	2.0 (0.9)%	0.5 (0.7)%
Wang (2002) MRI	Ventricles Brain	-8.2ml 2.14 (1.2)%	-0.8ml 0.4 (0.5)%
Jack (2003) MRI	Hippocampus	4.9 (2.1) %	-/-
Silbert (2003) MRI	Ventricles Brain	-5.5 (3.2)ml 17.9 (18.0)ml	-3.3 (3.5)ml 1.8 (9.6)ml

Figure 1.3 Registered coronal T1-weighted images of an individual with AD.



(A) Baseline image; (B) repeat scan registered to baseline; and (C) difference image (i.e. scan A subtracted from scan B) following rigid body registration

Figure 1.4 Outline of the steps required to quantify whole brain atrophy.



After acquisition of two serial volumetric MRI scans, both must be segmented (scalped) - stage 1. The two segmented scans are then digitally matched using a rigid-body registration - stage 2. Quantification is performed using the brain boundary shift integral which sums voxel shift between scans 2 and 1 at every brain:CSF boundary - stage 3.

2. Methods

2.1 Patient selection and inclusion criteria

2.1.1 Familial AD pedigrees

Pedigrees with a strong family history of Alzheimer's disease were identified from the records of the Dementia Research Group (DRG), St Mary's Hospital, and the National Hospital for Neurology and Neurosurgery, London, UK. The initial referral source of the families varied, some were collected following clinical or research referral of patients with a family history of dementia to Professor Martin Rossor, whilst other affected and unaffected individuals with a strong family history had contacted the DRG directly to enrol in research projects. Families were identified where the mode of inheritance appeared to be autosomal dominant and where the diagnosis of FAD in the pedigree was not in doubt (e.g. due to pathological confirmation in a previous generation). The mean age at onset (AAO) in each family was <61 years. In fourteen families mutations in the amyloid precursor protein (*APP*) or presenilin-1 (*PSEN1*) gene had been identified prior to commencement of the study. During the study further pathological mutations were identified in several other families.

Recruitment for clinical, neuropsychological, biochemical and MRI studies is described below by subject categories.

2.1.2 At risk subjects

Asymptomatic at-risk members of FAD pedigrees were asked to participate in the longitudinal study if they were within five years of the historical age at onset for their family. At recruitment a careful history for the presence of symptoms was obtained independently from the subject and their spouse or other close family member. Subjects were excluded from the at risk cohort if they already fulfilled NINCDS-ADRDA criteria for possible or probable AD. Subjects were only included if both they and other informants felt they had no symptoms of cognitive decline. Inclusion also required a close informant (usually a family member) who could provide a reliable independent history. At risk subjects were also recruited for plasma amyloid β (Aβ) peptide studies as described in Chapter 5.

2.1.3 Control subjects

The normal control subjects for the longitudinal study consisted of neurologically healthy individuals without a family history of early onset AD or other dementia. The control subjects were largely recruited from the spouses of affected or at risk study members. Control subjects were also considered for Aβ studies as described in Chapter 5.

2.1.4 Sporadic and familial AD subjects

Patients who already fulfilled NINCDS-ADRDA criteria for probable AD were also studied. These patients were recruited from the specialist cognitive disorders clinic at the National Hospital for Neurology and Neurosurgery. Both individuals with FAD and those without a strong family history of AD, sporadic AD (SAD), were included. All individuals had been fully investigated prior to recruitment. The investigations included screening blood tests for the reversible causes of dementia, brain MRI, EEG, Neuropsychology and CSF examination. The FAD subjects were also considered for genetic studies as detailed in Chapter 3. Both FAD and SAD subjects were also considered for Aβ studies as described in Chapter 5.

2.2 Consent and ethical considerations

The studies described in this thesis were approved by the local research ethics committees at The National Hospital for Neurology and Neurosurgery, Queen Square and St Mary's Hospital, Praed Street, London, UK. Informed written consent for each individual study was obtained from all study subjects or their appropriate legal representative. In addition written consent was obtained for each MRI scan. Individuals were not scanned if they had a contraindication to MRI such as a cardiac pacemaker. Although travelling expenses were reimbursed study subjects received no other payments or inducements. All subjects were informed that participation was entirely voluntary. They were also informed that they could at any time withdraw from the study and that withdrawal would not in any way influence any subsequent clinical care. Additionally for the longitudinal study a missed assessment would not preclude them from rejoining the study at a later date.

All individuals, and in particular the "at risk" cases, were made aware that the data collected as a part of the study were confidential and of a research nature. As part of the process of informed consent it was emphasised that the study would not provide any specific results on an individual basis. The one exception to this rule was if an MR scan revealed an unrelated but clinically important structural lesion such as a cerebral neoplasm in which case they and their general practitioner would be informed of the scan results. It was stressed that because of the experimental nature of the MRI analysis technique it would not be possible to provide information as to whether or not there was a suggestion of the onset of a degenerative process. It was however, made clear that if individuals or their relatives were concerned that they might be becoming symptomatic they could be referred to the National Hospital for evaluation in a clinical setting and further investigation as appropriate. All such referrals were accompanied by counselling. During the course of the study several subjects chose to be referred for these purposes.

For the genetic study described in Chapter 3 consent for research screening was obtained on the basis that there would be no individual results available. The consent obtained allowed family members to be informed of positive mutation findings for each specific family. These results were mailed using a standard letter to registered family members and their general practitioners without making individual results available to that family. It was made clear that these research findings would have to be confirmed in a clinical setting. This practice led to a small number of asymptomatic at risk individuals of FAD requesting genetic testing. In these cases referral for genetic counselling for consideration of predictive genetic testing was suggested via their general practitioner to either their local Clinical Genetics Service or to Professor Nick Wood, consultant neurogeneticist at The National Hospital for Neurology and Neurosurgery. In either case any information to assist in the genetic counselling process was made available to the clinician on request. In addition an offer to perform the actual genetic test under confidential clinical conditions by Professor John Collinge, Director of the MRC Prion Unit was always made as FAD mutation screening is not generally available in the UK. The finding of pathogenic mutations also allowed diagnostic genetic testing in patients who fulfilled NINCDS-ADRDA criteria.

In view of the stresses and anxieties which at risk individuals and their spouses experience and the fact that research assessments may exacerbate those

stresses, every effort was made to provide time for subjects in addition to that necessary for the research assessments. This allowed discussion of their concerns and provision of general information on AD and research being carried out. Members of families were also offered access to CANDID (counselling and diagnosis in dementia) a national telemedicine service supporting the care of younger patients with dementia run from the National Hospital for Neurology and Neurosurgery (Harvey *et al.* 1998b). Many of the subjects stated that they did not expect their participation in a research project to benefit them personally but hoped that they might help future generations.

2.3 Clinical and neuropsychological assessment

2.3.1 Assessment schedule

Subjects who fulfilled the inclusion criteria for the longitudinal study and who agreed to participate were asked to attend assessments accompanied by their spouse or a close family member. Study visits were scheduled to take place at intervals of approximately one year. However, if an individual was unable to make an assessment this did not exclude them from further study. Each assessment for the longitudinal study consisted of an interview, neurological examination, neuropsychological assessment and brain MRI. In addition blood samples for plasma Aβ studies were taken. The accompanying relative was interviewed separately to corroborate the history and to discuss their concerns about the disease. Many individuals travelled considerable distances to take part in the study and therefore the total assessment time was designed to allow travel and the assessment to be completed within the day.

2.3.2 History and examination

The Medical Research Council (UK) guidelines for assessment of AD research patients provided the basis for the clinical interview (Medical Research Council 1986). Specific questions covered activities of daily living, occupational history, and whether there had been any recent difficulties in coping with the demands of the job or home. Memory functions that were specifically enquired about included difficulties in remembering names, appointments, routes, telephone messages, errands or lists of items such as shopping. The subject and informant were independently asked by me whether there had been any change in memory functions and how the subject's

memory compared to that of other individuals of a similar age. Enquiries were also made about any changes in other cognitive functions or in behaviour. In addition to basic demographic data about the subject a full general medical and psychiatric history was obtained including alcohol, drug and tobacco consumption. Details of the family history were corroborated and the estimated age at onset of symptoms in affected family members verified. A standardised (modified) Mini Mental State Examination (see Appendix 2), and the Clinical Dementia Rating (CDR) (Hughes *et al.* 1982) were recorded for each visit for all subjects. A general neurological examination was performed on all subjects which included assessments of tone, tendon and primitive reflexes, myoclonus and praxis.

2.3.3 Neuropsychology

A comprehensive assessment was carried out by a neuropsychology assistant under the supervision of Dr Lisa Cipolotti, Head of the department of Neuropsychology, National Hospital for Neurology and Neurosurgery. The following areas of cognition were investigated: General intellectual functions (Shortened version of the WAIS-R) (Wechsler 1981), Advanced Progressive Matrices (Raven 1965), Verbal recognition and recall memory (Verbal version of the Recognition Memory Test (Warrington 1984), Paired Associate Learning Test (Warrington 1996), Visual Memory (Visual version of the Recognition Memory Test (Warrington 1984), Topographical memory test (Warrington 1996), Nominal function (Graded-difficulty naming test (McKenna & Warrington 1980)), Word comprehension (Synonyms test (Warrington, McKenna, & Orpwood 1998)), Literacy and calculation using the National Adult Reading Test (NART) (Nelson & Willison 1991), Graded-Difficulty Spelling Test (Baxter & Warrington 1994), Graded-difficulty Arithmetic Test (Jackson & Warrington 1986), Visual perceptual and visuospatial functions (Silhouettes and Cubes from the Visual Object and Space Perception Battery (Warrington & James 1991), Frontal 'executive' functions (Modified Card Sorting Test, (Nelson 1976), Cognitive Estimates test, (Shallice & Evans 1978), Trail Making test (Reitan 1958), Hayling sentence completion test (Burgess & Shallice 1996), Weigl colour form sorting test (Weigl 1948), Speed and attention (Digit copying and Cancelling 0's (Willison & Warrington 1992).

The assessment schedule was designed as a compromise between being as short as possible and yet being comprehensive. This test battery takes between 90 and 120 minutes to administer. Tests were chosen that were of graded difficulty and on which normal subjects rarely score at ceiling. So as to allow measurement of change the NART (or Schonell in severely affected individuals) was used to provide a measure of optimum level of pre-morbid intellectual function (Nelson & Willison 1991).

2.3.4 Blood sampling

For genetic studies three plastic 10 ml EDTA tubes were drawn using a standard aseptic Vacutainer® technique and either taken directly to the laboratory for DNA extraction or frozen at -70°C for subsequent DNA extraction. For the plasma Aβ studies two EDTA tubes were drawn and immediately centrifuged at 4000 rpm for three minutes. As soon as the centrifuge had stopped the supernatant was pipetted into 1 ml plastic aliquots without disturbing the buffy coat. These aliquots were immediately "fast" frozen using dry ice and transferred to a -70°C laboratory freezer for later analysis.

2.4 Magnetic resonance imaging

2.4.1 Acquisition

MR imaging was performed on a General Electric 1.5 Tesla Signa Advantage Unit (GE, Milwaukee, USA) at one of three sites: St. Mary's Hospital, Paddington, the NMR Research Unit at the Institute of Neurology or Queen Square Imaging Centre at the National Hospital for Neurology. All scans included a routine sagittal (T1-weighted) localizer sequence and an axial dual-echo sequence (T2- and proton density-weighted). In addition volumetric imaging was performed in the coronal plane, using a spoiled gradient echo technique (SPGR) with a 24 x 24 x 19.2 cm field of view yielding 124 contiguous 1.5 mm thick slices through the head on a 256*128 image matrix. The acquisition parameters were as follows: time to echo, 5 ms; time to repeat 35 ms; flip angle 35° (35/5/1/35 - TR/TE/NEX/FLIP).

Subjects were reminded to remain as still as possible during the scanning and were given earplugs to wear. They were positioned supine on the scanner table and head movement restricted by foam wedges either side of the head and a Velcro® band

across the forehead. Despite these restraints head movement remains possible and their function is mainly to support the head and to remind subjects to keep still. For comfort a foam wedge was placed under the knees to keep them partly flexed. For anxious at risk or affected subjects I remained in the scanner suite within the visual field of the head coil mirror for reassurance and to remind them to keep still. Sedation was not used. The duration of acquisition for the volumetric SPGR sequence was nine and a half minutes.

2.4.2 Image analysis tools

Digitised MR images were transferred to a Sun work station (Sun Microsystems Inc., Mountain View, CA) and analysis performed using the DRG in-house MIDAS image analysis programme (Freeborough, Fox, & Kitney 1997). The programme allows the 3-D data to be visualised in two windows simultaneously showing different orthogonal views (i.e. any two of axial, coronal, or sagittal planes). A 2-D cut of the data is displayed in the main window and the region of interest is defined upon it, using a mouse driven cursor to draw around an area and deposit a seed within it. The outlined region follows pixel boundaries. The outlining may be performed at a range of magnifications, and the level of magnification may be changed at any stage without any loss of data. Thresholding based upon grey level (signal intensity) may also be used to facilitate segmentation. The region of interest appears as it is defined, in real time, on orthogonal 2-D cuts displayed in the adjacent MIDAS window. In this way the relationship between the outlined region and neuroanatomical landmarks used to guide the outlining can be viewed in different planes simultaneously. This improves reproducibility of anatomical definition. The outlining may be edited in any other plane and corrected if it does not coincide with anatomical landmarks. Measurements may be outputted as slice-by-slice cross-sectional area measurements or as a volume. All regions can be saved and corrected at a later date.

2.4.3 Registration of serial MRI

Automated image subtraction allows all areas of the brain to be examined simultaneously without laborious manual measurements and *a priori* decisions of predetermined structures. However accurate positional matching must be achieved in

order for subtraction of serial studies to be meaningful. Careful standardised head alignment within the scanner (Bergstrom *et al.* 1981; Hajnal *et al.* 1995; Strother *et al.* 1994) is not sufficient because of the changing position of the atrophying brain relative to the skull interior. This has resulted in the development of post-acquisition positional matching or registration algorithms to align regions of interests on the baseline and follow-up studies. In this thesis registration was achieved using signal intensities from all voxels within the brain so that voxels representing the same underlying anatomical structures are superimposed. The DRG semi-automated morphological approach was used to define the brain region (Freeborough, Fox, & Kitney 1997; Freeborough, Woods, & Fox 1996). This interactive method uses conditional morphology in a way that minimises the distortion of structure and results in consistent placing of boundaries on different scans, features that are particularly important when dealing with the atrophied brain. The method is tailored for T_1 weighted MRI scans (Freeborough, Fox, & Kitney 1997; Freeborough, Woods, & Fox 1996). The primary tissue types, in such scans, of white matter (WM), grey matter (GM) and cerebro-spinal fluid (CSF) have high, intermediate and low voxel intensities respectively. The steps (see Appendix 3 for description and illustrations) involve selecting: 1) a range of intensities for brain tissue and a level at which to cut the spinal cord from the brain, 2) a number of conditional erosions and an intensity threshold for each, which prevents erosion of voxels with higher intensity (note: after each erosion voxels which are not part of the largest connected set are discarded), 3) a number of dilations conditional on voxel intensities being within 60%-160% (arbitrary) of the mean intensity over the set and 4) a sampling factor, r, used to define an 'interior' of the brain over which the 60%-160% criteria is reapplied. Manual editing can be added where necessary.

Registration using this method appears most robust when only brain regions are registered (excluding non-brain voxels which may move relative to brain) and a multi-resolution approach is adopted. Scaling changes (voxel size inconsistencies) have consistently been an important source of error on the three scanners used. Registration was improved when these were compensated for. These methods achieve consistency of registration to subvoxel level both in controls and patients with neurodegenerative disorders (Freeborough, Fox, & Kitney 1997; Freeborough, Woods, & Fox 1996).

2.4.4 Quantification of volume change from registered MRI

Once the scans are accurately registered points in the scans may be compared directly. This may be done by simple subtraction or alternatively areas of change may be highlighted on the scans using a *difference overlay image* (DOI); as illustrated in Figure 8.1. A DOI gives a magnified view of one scan on which pixels of *significant* change are coloured using alternate pixels to add colour without losing structural detail (Fox, Freeborough, & Rossor 1996). An increase in pixel intensity is represented by green and a decrease by red. Qualitatively the amount of change between scans may best be appreciated visually by toggling between scans or by displaying the red and green overlay to identify voxels of significant intensity change. Objective quantification of change was achieved using the Brain Boundary Shift Integral (BBSI), which automatically determines the total volume through which the whole boundary of a 3-D structure shifts from the voxel intensities of accurately registered serially acquired scans (Fox, Freeborough, & Rossor 1996).

2.4.5 Ventricular segmentation

The protocol that I used for ventricular segmentation is described in detail in Appendix 4, but in essence involves the semi-automated labelling of the region of interest using a set of "rules". Once labelled on one scan it can be "copied and pasted" onto the next of a pair of registered scans and edited. This minimizes changes in the application of the "rules" within individual patients.

3. A study of the genetics of familial Alzheimer's disease

3.1 Family review

Against a background of only fourteen DRG FAD families having an identified causative mutation (see Table 3.1) at the start of this project I reviewed the collection of families to identify potential probands for genetic screening using the following criteria:

1. Probable or definite AD using NINCDS-ADRDA criteria
2. Mean age of onset 60 years or less
3. Family history:
   - autosomal dominant inheritance defined using recognised criteria as having at least three affected members in two or more generations
   - affected relative(s), but not fulfilling recognised criteria for autosomal dominant inheritance
4. Availability of stored blood, stored DNA, frozen brain tissue or living affected family member involved in research project/ clinical follow up from whom a sample could be drawn with appropriate consent
5. Not known to be related to a DRG family with a known mutation

The majority of probands identified belonged to families that had previously been screened and no mutation found (e.g. F53, F105, F134, F160, and F175 who were reported as not having *APP* mutations by Fidani *et al.* in 1992). As shown in Figure 3.1, two thirds of DRG FAD families did not have an identified mutation raising the possibility that there might be a fourth AD locus.

In order to determine the proportion of DRG FAD families accounted for by mutations in known genes a systematic genetic evaluation was carried out by sequencing *APP*, *PSEN1* and *PSEN2*.

segregation between the mutation and the disease. In many families, however, this analysis was not possible, since there were no other living affected relatives/or stored DNA available. In addition 100 normal Caucasian controls were genotyped to determine the prevalence of a novel finding in the normal population. A standard one stage PCR technique was used to determine APOE genotype for probands in whom a causative mutation was not identified (Wenham, Price, & Blandell 1991).

3.3 Results

Seventeen patients with probable and 14 patients with definite AD (NINCDS-ADRDA criteria) were recruited. The mean AAO was 46.9 years (median: 45 years; range: 33-60 years). The majority of patients (23/31) belonged to families with three or more affected family members in at least two generations and thus had a family history fulfilling recognized criteria for autosomal dominant inheritance. The remaining eight patients had a single affected first degree relative; for six patients this was a parent and for two patients a sibling.

As shown in Table 3.4 sixteen *PSEN1* mutations were identified in 17 (55%) probands including eight recognised *PSEN1* mutations: Y115C, M146I, L153V, L171P, G184D, A260V, R269H and the intron4 or Δ4 mutation (Thr 113/114 ins), a novel three base pair deletion (Δ167) and seven novel missense point changes: Y154C, I229F, F237L, L235V, C263F, R377M and G378V. The Δ167 deletion was characterized by the deletion of the third base pair of codon 167 and the first and second base pair of codon 168.

As shown in Table 3.3 three *APP* mutations were identified in four (13%) probands, including the recognised V717I (London) mutation in two probands and two novel missense point mutations: V715A and H677R.

All novel mutations identified were absent from 100 healthy unrelated white control patients. In three families DNA was available from affected relatives. In these three families co-segregation of the mutation and the disease could be tested. In families 134 (*PSEN1* G378V) and 267 (*PSEN1* L235V) the presence of the sequence variant was demonstrated in all affected patients for whom DNA was available. In familiy 134 the G378V mutation was present in three patients, and in family 267 the L235V mutation was present in two patients. However, in family 209 the *APP* H677R

mutation was absent from the affected sibling; both siblings were APOE ε3 homozygotes. Thus in this series 21/31 (68%) probands were associated with a mutation in *APP* or *PSEN1*. For the subset of patients with definite AD who also fulfil criteria for autosomal dominant inheritance 9/11 (82%) probands were associated with a mutation in *APP* or *PSEN1* (five recognized mutations, two mutations that co-segregate with disease and two novel mutations only demonstrated in a single proband). Similarly 10/13 (77%) probands with probable AD (NINCDS-ADRDA), who also fulfilled criteria for autosomal dominant inheritance, were associated with a mutation in *APP* or *PSEN1* (seven recognized mutations and three novel mutations only demonstrated in a single proband).

The mean age of probands with mutations was 44.7 years, whereas those without mutations were significantly older at 55.4 years (P = 0.001). The mean age of probands with *PSEN1* mutations was not significantly younger than those with *APP* mutations (43.7 years v. 50.3 years; NS).

In 10 probands a mutation was not identified in *PSEN1* or the examined exons of *APP* and *PSEN2*; three probands were homozygous and five patients were heterozygous for *APOE* ε4 (see Table 3.5). The frequency of the *APOE* ε4 allele was 0.80 in these probands without mutations.

3.4 Discussion

Mutational analysis showed that 21/31 probands in this UK cohort had *PSEN1* or *APP* mutations. In addition *PSEN1* E318G, a non-causative rare polymorphism (Dermaut *et al.* 1999), was identified in family 181.

Evidence for the co-segregation of FAD and the novel mutations was only available in two families and so the fact that some of the novel mutations are non-pathogenic cannot be excluded. However, the pathogenicity of *PSEN1* and *APP* mutations is well recognised. Mutations in *APP* are located close to the major APP processing sites, either adjacent to the Aβ domain (the β and γ secretase cleavage sites) or within the Aβ domain itself (the α secretase cleavage site). The pathophysiological mechanisms of these mutations vary. The only known mutation near to the β secretase cleavage site is the 670/671 (Swedish) double mutation which consists of the substitution KM → NL. This mutation results in increased total Aβ, by increasing both $A\beta_{40}$ and, to a lesser extent, $A\beta_{42}$ (Scheuner *et al.* 1996). Those near the γ secretase cleavage site generally increase production of the more amyloidogenic

$A\beta_{42}$ (Scheuner *et al.* 1996). However, the V715M (French) mutation results in a reduction of $A\beta_{40}$ without affecting $A\beta_{42}$ production suggesting that it is the increase in the ratio of $A\beta_{42}$ to $A\beta_{40}$ which is important rather than the absolute amount of $A\beta_{42}$ (Ancolio *et al.* 1999). The V715A *APP* mutation described in this thesis is also at this residue. The neuropathology of the proband is typical of AD, but due to the lack of DNA specimens from further affected relatives co-segregation with disease could not be demonstrated. A more recent study of six mutations near the γ secretase cleavage site not only confirmed the findings of Ancolio *et al.* (1999), but also confirmed this for the V715A mutation (demonstrated in a German pedigree without neuropathology), and further demonstrated an inverse correlation between these ratios and AAO (De Jonghe *et al.* 2001).

Pathogenic intra-Aβ mutations (see Figure 1.1) are generally associated with amyloid accumulation in cerebral blood vessels in addition to amyloid plaque formation. Carriers of the E693Q (Dutch) mutation have intracerebral haemorrhages (Levy *et al.* 1990), whereas patients with the A692G (Flemish) mutation, frequently have intracerebral haemorrhage but may survive to develop a progressive AD-like dementia (Hendriks *et al.* 1992). The E693G (Arctic) mutation is associated with a more typical early onset AD phenotype without evidence of intracerebral hemorrhage. The three known mutations at codon 693 (see Figure 1.1) result in reduced $A\beta_{42}$ levels in conditioned media, which contrasts with increased levels of both $A\beta_{40}$ and $A\beta_{42}$ in media for the A692G (Flemish) mutation (Nilsberth *et al.* 2001). For the E693G (Arctic) mutation this reduction in Aβ was observed even in pre-symptomatic mutation carriers 20-30 years before the expected onset of the disease. This together with the finding that Arctic Aβ formed protofibrils at a much higher rate and in larger quantities than wild type Aβ is the basis of a proposed alternative pathogenic mechanism for this kindred where protofibrils result in the accelerated buildup of insoluble Aβ (Nilsberth *et al.* 2001). The pathogenicity of *APP* H677R, the novel intra-Aβ sequence variant which was present in one of two siblings of family 209, is not certain. The diagnosis in both siblings is secure since they both underwent neuropathological examination which confirmed the signature lesions of AD. Neither sibling had an *APOE* ε4 allele, a mutation in *PSEN1* or the sequenced exons of *PSEN2* to account for their disease. Nothing is known about the previous generation in this family so it remains a possibility that the mutation is an autosomal dominant pathogenic mutation accounting for the disease in one of the siblings with the other

sibling being a phenocopy i.e. having sporadic AD. Alternatively it is a rare non-pathogenic polymorphism, absent from 100 normal Caucasian controls, and both siblings have sporadic AD. Further structural modelling and Aβ metabolism studies for this novel sequence variant are awaited.

Of the seven novel *PSEN1* missense mutations in this study two occur at residues where mutations have previously been reported: C263F and G378V (Besancon *et al.* 1998; Wasco *et al.* 1995). In keeping with previous reports the remaining five missense mutations occur in transmembrane domains (TM). The Y154C mutation further extends the previously discussed mutation cluster in TM 2 in exon 5 (see Table 4.6). The I229F and F237L mutations are in TM 5 in exon 7 (Aldudo, Bullido, & Valdivieso 1999; Campion *et al.* 1995a; Campion *et al.* 1995b; Campion *et al.* 1999; Cruts *et al.* 1998; Kwok *et al.* 1997). The L235V mutation lies in a small cluster in TM 6 in exons 6/7 (Forsell *et al.* 1997; Hutton *et al.* 1996; Rogaev *et al.* 1995; Sherrington *et al.* 1995m). The R377M mutation is in the TM7 cluster in exon 11 (Besancon *et al.* 1998; Campion *et al.* 1995b; Campion *et al.* 1999; Cruts *et al.* 1995; Tedde *et al.* 2000).

The pathogenicity of *PSEN1* deletion mutations is also well recognised (Crook *et al.* 1998; Kwok *et al.* 1997; Steiner *et al.* 2001), and though critically dependant on what is actually deleted, the novel *PSEN1* Δ167 mutation is probably also pathogenic.

Mutations of *APP* and *PSEN1* show almost complete penetrance by the age of 60 years with the exception of four *PSEN1* missense mutations: A79V (Cruts *et al.* 1998), I143F (Rossor *et al.* 1996), H163Y (Axelman, Basun, & Lannfelt 1998), and H163R (Poorkaj *et al.* 1998b). The level of penetrance and mechanism of pathogenicity remain to be determined for the novel mutations described here. No mutations were identified in exons 4, 5, and 7 of *PSEN2* which may reflect the selection of families with an early and relatively constant AAO.

The proportion of *PSEN1* and *APP* mutations described in the present study is much higher than the 18% reported by Cruts *et al.* (1998) who used the same criteria to define autosomal dominant inheritance. The findings from this study are in accord with a French FAD study in which 24/34 (71%) families fulfilling stricter three generational criteria to define autosomal dominant inheritance were found to have mutations in *APP* or *PSEN1* (Campion *et al.* 1999).

Ten probands did not have a mutation in *APP, PSEN1* or *PSEN2*. Despite there being two very early onset probands (families 278 and 373) in this group it is of

interest to note that the mean age of these probands was ten years older than those with mutations (P = 0.001) and that, three (30%) of these probands were homozygous and five (50%) were heterozygous for *APOE* ε4. Thus the observed frequency of the APOE ε4 allele of 0.80 was higher than the 0.15 observed in the general Caucasian population (Campion *et al.* 1999; Weiner *et al.* 1999). In view of their older AAO it is plausible that some of these patients may in fact have early onset AD due to the presence of one or two ε4 alleles. The proband from family 278, with a three generational family history, did not have an ε4 allele, and the absence of an *APP*, *PSEN1* or *PSEN2* mutation in this neuropathologically confirmed family, could be explained by a mutation located outside the regions that were analysed, or by the involvement of other causative and risk genes for FAD. Although the proband from family 373 was homozygous for ε4, it seems unlikely that this is *APOE* driven AD and again a mutation outside the regions analysed or the involvement of another genetic factor seems more likely.

Familial AD is a rare disease with an estimated prevalence of 5.3 persons per 100,000 at risk (Campion *et al.* 1999). The data from this study are therefore from a highly selected population of patients who have been carefully characterised and fulfil either ante-mortem NINCDS-ADRDA criteria for a diagnosis of probable AD or have neuropathologically verified AD. Eighty-one percent of FAD patients (9/11) with neuropathologically confirmed definite AD had an associated mutation in either *APP* or *PSEN1*. Six of these mutations have been previously reported, two were novel changes for which co-segregation was demonstrated, and one was a novel change demonstrated in the proband only. Seventy seven percent of probable FAD probands (10/13) had associated mutations in *APP* or *PSEN1*. Seven of these mutations were recognised pathogenic mutations and three were novel mutations demonstrated in a single proband only. It is these novel mutations only demonstrated in a single proband that cause the greatest difficulties in clinical practice, particularly for *PSEN1* mutations as many are private, i.e. they are only found in a particular individual or family (Blacker & Tanzi 1998; Cruts *et al.* 1998). It is therefore difficult to interpret the causative effects of the novel mutations described and this will require extending the families where possible, looking at the pathophysiology of the mutations with regards their effect on APP processing and waiting for further reports.

Because a molecular genetic diagnosis of an inherited disorder affects not only the patient, but also the entire family, genetic counselling must be an essential

component of the diagnosis of inherited disorders (Gasser *et al.* 2001). In the case of predictive testing this will invariably involve trained clinical geneticists using a (modified) Huntington's disease protocol. The observed mutation frequency of 78% in 23 FAD probands in this study suggests that a molecular diagnosis is well worth pursuing in a patient with early onset probable AD. On the whole these data and the literature (Campion *et al.* 1999; Cruts *et al.* 1998; Finckh *et al.* 2000) would suggest that this should be confined to those with a clearly positive family history of autosomal dominant inheritance and an early AAO in all family members. Mutational screening of affected individuals without such an autosomal dominant family history is rarely indicated unless there is a very characteristic phenotype and inadequate family history (Gasser *et al.* 2001). This would certainly apply to the two EOAD patients with an AAO in the fourth decade of life with *PSEN1* mutations and possibly the sib-pair of F209, although both non-paternity and the remote possibility of a *de novo* mutation should also be considered. Routine counselling prior to diagnostic testing would include making the patient and the family aware that a mutation may not be found and that a novel finding may be difficult to interpret.

In a clinical setting, however, the emphasis must remain on making an accurate clinical diagnosis and excluding the many reversible conditions that can masquerade as AD. In addition it should not be forgotten that families who do not have a proven pathogenic mutation or who do not wish to undergo diagnostic genetic screening should not be denied the benefits of genetic counselling based on the autosomal dominant risk model. Even for those with a known pathogenic mutation the uptake of predictive testing may be low, similar to Huntington's disease (Binedell, Soldan, & Harper 1998), in the absence of a disease modifying treatment.

Following the completion of this study, and the finding of the intron 4/Δ4 mutation in F105/160 and F175 (De Jonghe *et al.* 1999b) the combined post-study DRG FAD mutation prevalence is 88% (as illustrated in Figure 3.2). Though the likelihood of a fourth FAD locus existing would appear to be much diminished several large kindreds e.g. F278 and F373 are yet to be accounted for in this series and we are sequencing the remaining exons of *PSEN2* and reviewing the pathology of these non-mutation FAD families, prior to embarking on further studies.

Table 3.1 DRG FAD families with known mutations in 1998

Family	Gene	Mutation	First Author
19	*APP*	V717G	Chartier-Harlin, 1991
23	*APP*	V717I	Goate, 1991
74	*PSEN1*	Δ9 S290C	Perez-Tur, 1996
102	*PSEN1*	E280G	Clark, 1995
121	*PSEN1*	E120K	Hutton, 1996
148	*PSEN1*	M139V	Clark, 1995
156	*PSEN1*	I143F	Palmer, 1999
168	*PSEN1*	E280G	Clark, 1995
172	*APP*	V717I	Fidani, 1992
183	*PSEN1*	E280G	Clark, 1995
184	*PSEN1*	L250S	Hutton, 1996
196	*PSEN1*	P267S	Clark, 1995
206	*PSEN1*	M139V	Clark, 1995
223	*PSEN1*	P436S	Palmer, 1999

APP = amyloid precursor protein gene mutation

PSEN1 = presenilin 1 gene mutation

Table 3.2: Mutations demonstrated and method of confirmation

Family	Gene	Mutation	Controls	Confirmation Assay	ASOH probe Wild type/mutant
36	*APP*	V717I	-	ASOH	AGTGATC**G**/**A**TCATCAC
37	*PSEN1*	F237L	100	ASOH	CCTGGTG**T**/**C**TTATCAA
53	*PSEN1*	Δ167	100	ASOH	GCTTATTATATCATC/ TGGCTTATATCATCT
134	*PSEN1*	G378V	100	ASOH	TTAGGGG**G**/**T**AGTAAAA
177	*PSEN1*	L153V	100	ASOH	GGTGGTT**C**/**G**TGTATAA
181	*PSEN1*	E318G	-	ASOH	AATGCAGAAAGTAGG/ AATGCAGGAAGTAGG
209	*APP*	H677R	100	*Nla III*	-
226	*PSEN1*	R269H	-	*Nde* I	-
250	*APP*	V715A	100	*Pvu* I	-
266	*PSEN1*	L171P	-	ASOH	TCATCTC**T**/**C**ATTGTTG
267	*PSEN1*	L235V	100	ASOH	CATGGCC**C**/**G**TGGTGTT
283	*PSEN1*	R377M	100	*Bsl* I	-
286	*PSEN1*	I229F	100	ASOH	CATTATG**A**/**T**TTAGTGC
301	*PSEN1*	C263F	100	ASOH	GTTTTGT**G**/**T**TCCGAAA
325	*PSEN1*	M146I	-	ASOH	TTGTCAT**G**/**A**ACTATCC
342	*PSEN1*	Intron4	-	*Pvu* II	-
344	*PSEN1*	Intron4	-	*Pvu* II	-
349	*APP*	V717I	-	ASOH	AGTGATC**G**/**A**TCATCAC
353	*PSEN1*	A260V	-	ASOH	TTAGTGG**C**/**T**TGTTTTG
364	*PSEN1*	E184D	-	*Afl* III	-
369	*PSEN1*	Y154C	100	ASOH	GTTCTGT**A**/**G**TAAATAC
372	*PSEN1*	Y115C	-	ASOH	AGAATCT**A**/**G**TACCCCA

ASOH = Allele specific oligonucleotide hybridisation

Table 3.3 Probands with APP mutations

Family	Affected Patients (n)	Generations (n)	AAO, Mean (range) years	Exon	Mutation	Amino Acid Change	PM
36	5	2	52, 50 (49-52)	17	V717I	Val717Ile	AD
209	2	1	55, 55 (55-56)	16	H677R	His677Arg*	AD
250	3	2	42, NK (NK)	17	V715A	Val715Ala*	AD
349	5	3	54, NK (NK)	17	V717I	Val717Ile	-

* Novel mutation

Table 3.4 Probands with PSEN1 mutations

Family	Affected Patients (n)	Generations (n)	AAO, Mean (range) years	Exon	Mutation	Amino Acid Change	PM
37	3	2	46, 51 (46-57)	7	F237L	Phe237Leu*	-
53	5	2	45, 52 (45-60)	6	Δ167	Δ167*	AD
134	13	3	43, 45 (41-49)	11	G378V	Gly378Val**	AD
177	3	2	35, 35 (35-36)	5	L153V	Leu153Val	AD
181	2	2	52, 61 (52-70)	9	E318G	Glu318Gly***	-
226	4	3	53, 53 (50-56)	8	R269H	Arg269His	AD
266	3	3	38, 38 (38-38)	6	L171P	Leu171Pro	-
267	6	3	44, 47 (44-50)	7	L235V	Leu235Val**	AD
283	2	2	38, 39 (38-41)	11	R377M	Arg377Met*	AD
286	2	2	33, 33 (33-33)	7	I229F	Ile229Phe*	AD
301	3	2	58, 58 (58-60)	8	C263F	Cys263Phe*	-
325	3	2	49, 49 (49-50)	5	M146I	Met146Ile	-
342	3	3	34, NK (NK)	IVS4	Intron4/ Δ4	Thr 113/114 ins	-
344	3	2	37, NK (NK)	IVS4	Intron4/ Δ4	Thr 113/114 ins	AD
353	6	2	40, 41 (40-42)	8	A260V	Ala260Val	-
364	5	3	42, 42 (40-44)	7	E184D	Glu184Asp	-
369	6	3	41, NK (NK)	5	Y154C	Tyr154Cys*	-
372	5	3	36, 48 (36-54)	5	Y115C	Tyr115Cys	-

* Novel mutation, ** Co-segregation demonstrated, *** Non causative polymorphism

Table 3.5 Probands without causative FAD mutations

Family	Affected subjects	Generations	AAO, Mean (range) years	*APOE* Genotype*	PM
112	2	2	55, 62 (55-69)	3,4	-
150	4	2	60, 61 (60-63)	4,4	-
157	4	2	58, 59 (58-60)	4,4	-
165	2	2	53, 56 (53-60)	3,4	AD
167	2	2	58, 58 (57-58)	3,4	AD
181	2	2	52, 61 (52-70)	3,4	-
278	13	3	47, 45 (42-48)	3,3	AD
287	5	3	60, 59 (58-60)	3,4	-
296	2	2	60, 60 (60-60)	3,3	-
373	5	3	36, 34 (32-36)	4,4	AD

AAO = age at onset,
PM = post mortem diagnosis
* Of the index patient

Figure 3.1 Mutation frequency in DRG FAD families in 1998

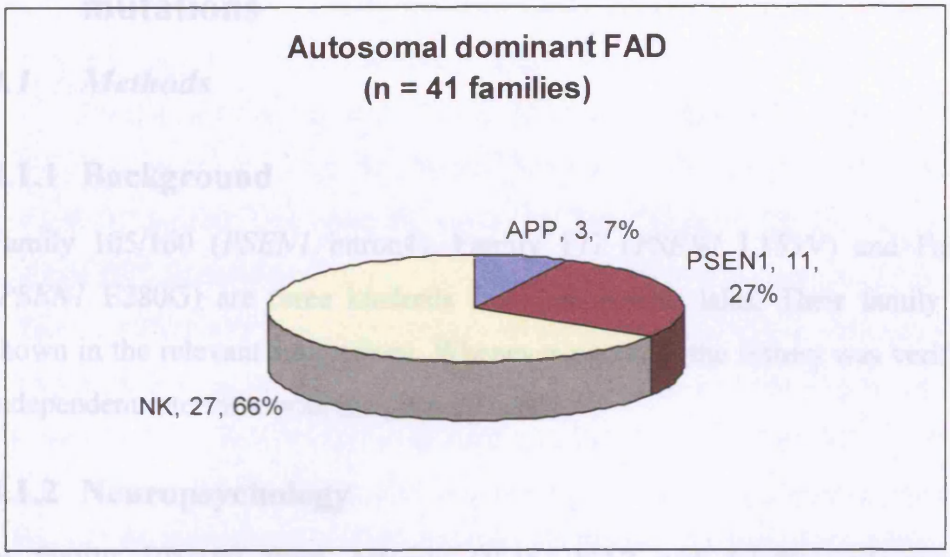
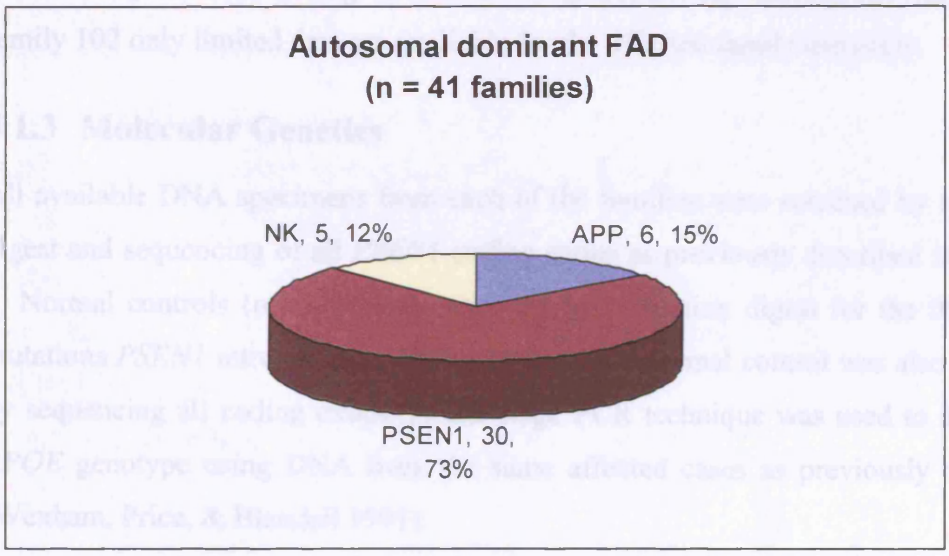


Figure 3.2 Mutation frequency in DRG FAD families in 2001



APP = amyloid precursor protein gene mutations
PSEN1 = presenilin 1 gene mutations
NK = not known

4. A study of the clinical phenotype of three *presenilin 1* mutations

4.1 Methods

4.1.1 Background

Family 105/160 (*PSEN1* intron4), Family 177 (*PSEN1* L153V) and Family 102 (*PSEN1* E280G) are three kindreds from the British Isles. Their family trees are shown in the relevant subsections. Whenever possible, the history was verified by an independent interview with the primary carer.

4.1.2 Neuropsychology

In family 105/160 three patients (V.16, V.17 and VI.08) underwent neuro-psychological assessment as described previously. A further patient (IV.15) had only been assessed on the Wechsler Adult Intelligence Scale (Wechsler 1955). In family 177 three members are enrolled in the longitudinal "at risk" study, but of the affected patients only III.3 had undergone a limited neuropsychology assessment. Similarly for family 102 only limited data are available for the affected family members.

4.1.3 Molecular Genetics

All available DNA specimens from each of the families were screened by restriction digest and sequencing of all *PSEN1* coding exons as previously described in Chapter 3. Normal controls (n >100) were screened by restriction digest for the then novel mutations *PSEN1* intron 4 and L153V. At least one normal control was also screened by sequencing all coding exons. A one stage PCR technique was used to determine *APOE* genotype using DNA from the same affected cases as previously described (Wenham, Price, & Blandell 1991).

4.1.4 Neuropathology

Neuropathological examinations were carried out in each of the three families. These were carried out by Professor Peter Lantos of the MRC Neurodegenerative Diseases Brain Bank at King's College Hospital on the following affected family members 105/160.V.10, 105/160.VI.08, and 177.III.3. Details of a further two necropsies

performed previously on patients 105/160.IV.11 and 105/160.IV.15 were also obtained. A brain biopsy had previously been performed on patient 102.III.2 and this was re-evaluated in detail by Professor Michael Farrell, Department of Neuropathology, Beaumont Hospital, Dublin.

Blocks of tissue from the brains of cases 105/160.V.10, 105/160.VI.08, and 177.III.3 were taken from the frontal, temporal (with the hippocampus and the amygdala), parietal and occipital lobes, the basal ganglia (to include the caudate nucleus, the lentiform nucleus, the claustrum and the nucleus basalis of Meynert), the thalamus with the subthalamic nucleus, midbrain, pons, medulla oblongata and from the cerebellar hemisphere and vermis. The sections were stained with haematoxylin and eosin, impregnated with silver according to the modified Bielschowsky method and luxol fast blue and cresyl violet (on selected sections). Antibodies were used to immunostain β-amyloid, glial fibrillary acidic protein (GFAP), tau protein (all from DAKO) and α-synuclein (kindly provided by Professor B H Anderton, Institute of Psychiatry, London).

4.2 Results

4.2.1 PSEN1 intron 4 mutation (Thr 113/114 ins)

PSEN1 intron 4, the only intronic PSEN1 mutation, was first reported in two individuals from a series of 40 cases of autopsy-confirmed early-onset AD that were all unselected for family history (Tysoe *et al.* 1998). It was subsequently demonstrated in four additional early onset FAD pedigrees including family 105/160 (De Jonghe *et al.* 1999b). The detailed clinical and neuropathological features of 21 affected individuals from family 105/160 are reported here. Figure 4.1 shows the family tree of family 105/160.

4.2.1.1 Family overview

Table 4.1 summarises the clinical features of family members. The mean age at onset was 37.4 years (95% CI: 36.7-38.2 years). The mean age at death was 44.7 years (95% CI:43.1-46.3 years). Disease duration was 7.3 years (95% CI: 5.9-8.7 years). Individuals I.1, II.1 and II.3 are known to have died at home following a dementing illness, no medical records survive. Cases III.1 to IV.08, V.07 and V.09 are all known to have died in the same local psychiatric hospital and to have had dementia. Their

clinical records were destroyed in a hospital fire. Ages at death were obtained from death certificates.

4.2.1.2 Case reports

IV.09. Age at onset 37 years; age at death 45 years. His first symptom was memory loss. He was eventually dismissed from his job, for continually wandering, one year before death. He had become increasingly childish, and was looked after by his family at home. He had frequent jerky movements and occasional generalised seizures. He became acutely confused one week prior to death having become violent and aggressive at home. He was admitted to the local psychiatric hospital. On examination he was disoriented in time and place. His speech was slurred. He was in poor physical condition. General examination was normal. His gait was reported to be ataxic and jerky movements, which were termed purposeless, were noted. He had symmetrical hyper-reflexia. Baseline blood tests and CSF examination were normal. The day following his lumbar puncture he developed acute dyspnoea, and died of bronchopneumonia. Postmortem examination confirmed bilateral pneumonia. The working diagnosis had been Huntington's disease, but the death certificate merely stated pre-senile dementia as the underlying cause of death. From the incomplete pathology notes the diagnosis of Alzheimer's disease had been considered. No formal histopathology data survive, other than the documented fresh brain weight of 1293 g.

IV.10. Age at onset 38 years; age at death 48 years. From the General Practitioner's records his first symptom was increasing forgetfulness, both at work and at home, and as a result he was given a less responsible job. He had considerable insight into his condition at the outset. He did not have chorea. Two months prior to death he was admitted to his local psychiatric hospital. Unfortunately the hospital records have been destroyed. His death certificate listed the following: Ia status epilepticus, duration two and a half hours. Ib cerebral degeneration, and II pre-senile dementia.

IV.11. Age at onset, 36 years; age at death 41 years. Only limited clinical details survive. This lady presented with symptoms of depression. Two years before death an EEG was mildly abnormal, but non-specific, with an 8 Hz dominant rhythm and some theta activity. She was pyrexial when admitted to hospital, and died of autopsy confirmed bilateral bronchopneumonia. Macroscopically the brain was

atrophied, most marked anteriorly, and "internal hydrocephalus" was noted. Sections for histology were taken from the left hemisphere, cerebellum and medulla oblongata. Routine staining with haematoxylin and eosin revealed changes consistent with AD. Plaques were most numerous in the frontal fragments. There was complete loss of cortical cyto-architecture, and significant neuronal degeneration, with the remaining neurones showing chronic degenerative changes, including a few cells with granulovacuolar degeneration.

IV.15. Age at onset 38 years; age at death 45 years. This lady presented to her local neurological hospital with at least a nine month history of progressive memory impairment. She had difficulty remembering names of familiar people and mild word finding difficulties were noted. She had considerable insight into her forgetfulness, and found it distressing. For one month prior to admission she had been having "sudden jerks" of the limbs. Lumbar air encephalogram showed evidence of cortical atrophy, with dilatation of the third ventricle and rounding of the lateral ventricles. Routine blood and CSF examination were normal. An EEG was abnormal, with reduced alpha activity in the posterior-central regions and theta activity in all regions. Non focal multiple spike and wave complexes were also seen. As she deteriorated she developed frequent generalised seizures and was admitted to a psychiatric hospital for full nursing care. She was treated with chlorpromazine, phenobarbitone and phenytoin. Two years later she died of pneumonia. Pathological examination (1969): revealed a brain weight of 964 gm with generalised cerebral atrophy but, relative sparing of the cerebellum and brainstem. Histology showed the characteristic features of AD. Recently performed ubiquitin immunostaining demonstrated many neurofibrillary tangles and neuritic plaques.

V.08. Age at onset, 40 years; age at death 44 years. This man presented at the age of 42 with a two year history of cognitive decline at work. He had worked as a trained instrument mechanic, but had had his responsibilities reduced because of memory and concentration problems. At the time of assessment he was working as an odd job man. On examination he was disorientated in time, and his digit span was three. It was noted that he showed a "peculiar type of motor restlessness", which was not clearly choreiform. At times he tried to disguise the movements by converting them into gesticulation. His wife had noted that the jerky movements were worse when he was dropping off to sleep. An EEG was abnormal and was thought to provide evidence of an organic dementia. A clinical diagnosis of Huntington's disease

was made because of the jerky movements and a strong family history of a mental disorder resulting in death at an early age. He died of pneumonia having been an in-patient in his local psychiatric hospital for a year.

V.10. Age at onset 39 years; age at death 54 years. This university graduate presented when he was made redundant from his teaching job for being unable to maintain discipline and forgetting staff meetings. At presentation there was a three year history of memory loss; he was fully aware of his family history and had preserved insight at the outset. General and neurological examinations were unremarkable; baseline dementia screening blood tests were normal or negative. Brain CT scan showed minimal cerebral atrophy. His EEG was abnormal with a low amplitude record with a symmetrical, regular and responsive alpha rhythm at 9-11 Hz. In addition there was a large amount of theta activity, which was maximal anteriorly. He deteriorated gradually, and his wife noted a personality change, with him becoming distrustful, and occasionally violent. There were times when he would not recognise her and thought that she was stealing their car. He became increasingly childlike, and began to wander. He neglected personal hygiene and was noted to have developed purposeless choreiform movements, particularly affecting the hands and arms, 7 and 8 years respectively into the disease. He became an inpatient 3 years later requiring full nursing care. Over the next eighteen months he continued to deteriorate, he became mute, and had his first generalised seizure, following which he was started on carbamazepine. Five years later he died of bronchopneumonia, neuropathological examination confirmed the diagnosis of Alzheimer's disease with amyloid angiopathy (see neuropathology section below).

V.12. Age at onset 40 years; age at death 45 years. The onset of this lady's symptoms coincided with a deterioration in the glycaemic control of her diabetes. A sudden deterioration in her memory and a change in her personality occurred after a severe hypoglycaemic episode accompanied by a generalised seizure two years prior to death. This resulted in a period of unconsciousness lasting for 11 hours. Since that time she had a clearly progressive dementing illness, and was noted to have myoclonus of the fingers and arms particularly, but also the legs and trunk. She had no further generalised seizures. She died in hospital of autopsy confirmed bronchopneumonia.

V.16. Age at onset 33 years. This right handed woman presented with a two year history of impairment in episodic memory and a change in personality; she had

become more introverted. She experienced word finding difficulty but otherwise continued to look after her family without significant problems. She had initially been treated for post-natal depression, but had failed to improve on tricyclic anti-depressant medication. There was no other past medical history, she did not smoke. Clinical examination revealed cognitive impairment which was confirmed on formal testing (see neuropsychology section and table 4.2), she scored 21/30 on the Mini Mental State Examination (MMSE) (Folstein, Folstein, & McHughs 1975). General physical examination was normal. Neurological examination revealed finger myoclonus. A pout and glabellar tap reflex were elicited. Baseline blood and CSF examination were normal or negative. Brain CT scan showed slight sulcal widening. An EEG revealed sporadic, symmetrical 8-9 Hz alpha rhythm over posterior regions and widespread 5-6 Hz irregular theta activity. Brain MRI, one year later, showed diffuse cortical atrophy with bilaterally small hippocampi. Examination now revealed Gegenhalten and mild dyspraxia. MMSE was 18/30. Two years later her myoclonus had become more marked and her MMSE had declined to 13/30. She made body part substitution errors during an assessment of praxis and had difficulty copying gestures with the left hand but not the right. She had suffered a single generalised seizure and had been started on sodium valproate. Four years after presentation there was evidence of further cognitive decline with speech and comprehension difficulties. The following year she had become increasingly dependent and required full time nursing care.

V.17. Age at onset 38 years. This right handed woman was referred to the Specialist Cognitive Disorders Clinic with a two year history of memory impairment. Her family commented that initially her conversation had become repetitive and she had increasing difficulties with remembering errands and telephone messages. Subsequently her culinary skills and the standard of her housekeeping had declined and she had started using lists to help organise her day's chores. Her MMSE was 27/30 (see neuropsychology section and Table 4.2 for cognitive profile). There was no past medical history of note. She was a non-smoker. Physical and neurological examinations were normal. Volumetric MRI brain scan was reported as normal (Figure 4.2A). A diagnosis of Alzheimer's disease was made. Repeat MRI brain scan 13 months later (Figure 4.2B) remained normal. When she was re-assessed at 24 months her MMSE had declined to 25/30. She had become more dependent in household activities. Volumetric MRI brain scan was repeated at 22 months (Figure

4.2C) and was again regarded as being within normal limits, however comparison with earlier scans showed progressive ventricular enlargement.

VI.08. Age at onset, 36 years; age at death 43 years. This left handed man, who had left school at the age of 16 years without formal qualifications, presented at the age of 38 years with at least an 18 month history of a progressive loss of mental agility. He had lost his job as a driver 30 months earlier, which was probably as a result of difficulties at work. There had been deterioration of his episodic memory and in particular with remembering the content of messages. More recently he had great difficulty with several practical skills. His family denied problems with route finding, faces or names. He was said to be independent in his activities of daily living. He had lost interest in reading, and spontaneous conversation was noted to be minimal. On examination his MMSE was 9/30, he was disorientated in time and place (see neuropsychology section and Table 4.2 for details). General physical and neurological examination was normal. Baseline blood investigations were normal or negative. MRI brain scan showed diffuse cerebral atrophy. EEG was abnormal with marked generalised slow (2-3 Hz) and theta (4-6 Hz) activity. There was modest attenuation of the slower components with eye opening. Over the next year he deteriorated further with evidence of visual disorientation, and finger myoclonus; he also developed generalised seizures, which were controlled with carbamazepine. He became increasingly aggressive. He was found wandering by the police, and admitted to his local psychiatric hospital where he remained an inpatient until his death 18 months later. An autopsy was arranged for brain donation (see neuropathology section).

4.2.1.3 Molecular Genetics

The PSEN1 intron 4 mutation (Tysoe *et al.* 1998) was demonstrated in the four affected individuals for whom DNA was available (cases V.10, V.16, V.17 and VI.08). It was absent from one unaffected member of F105/160 who was screened by restriction digest and sequencing of all coding exons. In addition two unaffected members and three non-consanguineous spouses were screened by restriction digest alone. Normal controls (n = 111) were screened by restriction digest, and one of these was also screened by sequencing all coding exons. The mutation was therefore.found to co-segregate with the disease. Linkage studies gave a LOD score of 3.72 and the mutation is thought to be fully penetrant.

The same four affected patients were *APOE* genotyped and these data are listed in Table 4.1. In one further deceased individual (case IV.15) it was possible to infer the APOE genotype from the APOE status of first degree relatives as her two affected children were respectively homozygous ε3/ε3 and ε4/ε4.

4.2.1.4 Neuropsychology

The neuropsychological assessments of four affected patients (IV.15, V.16, V.17 and VI.08) are summarised in Table 4.2.

General Intellectual Function

To obtain a measure of current intellectual functioning a shortened version of the Wechsler Adult Intelligence Scale-Revised (WAIS-R) (Wechsler 1981) was administered to all patients, except Case IV.15, who had been assessed on a shortened version of the WAIS. The WAIS IQ scores were converted into WAIS-R scores for comparison. The patients' premorbid optimum levels were estimated with the National Adult Reading Test (NART) (Nelson & Willison 1991). The discrepancy between the NART and the WAIS-R IQ scores provides a measure of the severity of intellectual deterioration.

At the time of their first assessment both IV.15 and V.16 presented with intellectual deterioration, affecting non-verbal IQ to a greater extent than verbal IQ. On subsequent assessments both patients showed increasing deterioration, affecting non-verbal functions to a greater extent. However, patient IV.15 presented a severe generalised decline on her third assessment. Patient V.17 showed mild intellectual decline on both measures, verbal scores being lower than performance scores. Patient VI.08 presented with global intellectual decline. Inspection of the cohort's age-scaled scores reveals that, in the verbal domain, the arithmetic and digit span subtests showed the greatest impairment in all cases, except V.17. In summary all patients had Verbal IQs that were higher than Performance IQs. In the verbal domain the arithmetic and digit span subtests were more affected than the vocabulary and similarities subtests.

Memory Skills

At the time of her first assessment patient IV.15 already had a severe and global memory impairment, which prevented formal assessment. Verbal and visual memory

functions were assessed with the Recognition Memory Test (RMT) (Warrington 1984) and the easy recognition memory test (Clegg & Warrington 1994) in patients V.16 and V.17, and with the shortened version of the Recognition Memory Test (CSRMT) (Warrington 1996) in patient VI.08.

At first assessment both patients V.16 and V.17 presented with selective verbal memory impairment. Subsequently patient V.16's memory functions became globally and severely impaired. In contrast case V.17 continued to present a selective verbal memory impairment. Case VI.08 presented with a severe and global memory impairment.

Naming Skills

Naming skills were assessed with the Graded Naming Test (GNT) (McKenna & Warrington 1983). Throughout assessments they remained well preserved in patients V.16 and V.17. Patient VI.08 obtained a poor score on the GNT, however, on the easier Oldfield Picture Naming Test (Oldfield & Wingfield 1965) he obtained a creditable score (22/30) in the context of his modest premorbid verbal functioning. Thus naming skills were well preserved in all patients across the assessments.

Literacy skills

Reading skills were assessed on the NART, spelling skills were assessed with the graded difficulty spelling test (GDS) (Baxter & Warrington 1994), and calculation skills were assessed with the graded difficulty arithmetic test (GDA) (Jackson & Warrington 1986). All patients demonstrated intact reading skills, which remained remarkably stable across assessments. In patients V.16 and V.17 spelling skills were preserved and did not deteriorate across assessments. Patient VI.08 obtained a poor score in the GDS, which in the context of his modest reading skills and relatively poor education, cannot be taken to indicate unambiguously a spelling impairment. Calculation skills were found to be severely impaired in all patients with the exception of V.17. In summary, reading and spelling skills were remarkably well preserved in two patients (V.16 and V.17), and did not deteriorate as the disease progressed. By contrast, calculation skills were impaired in two patients (VI.08 and V.16).

Perceptual and spatial skills

Three subtests of the Visual Object and Space Perception Battery (VOSP) (Warrington & James 1991), were administered to obtain a measure of perceptual (fragmented letters and silhouettes) and spatial skills (cube analysis). At first assessment patients V.16 and V.17 presented with preserved perceptual and spatial skills. Perceptual skills were found to be gravely impaired in patient VI.08.

Frontal executive functions

Frontal 'executive' functions were assessed using the Weigl test (Weigl 1948), the Wisconsin Card Sorting Test (Nelson 1976) and Verbal Fluency tests (Spreen & Strauss 1998) . Patients V.16 and VI.08 failed these tests. Patient V.17 completed the Wisconsin Card Sorting Test competently at her first assessment, but had become mildly impaired at her second assessment.

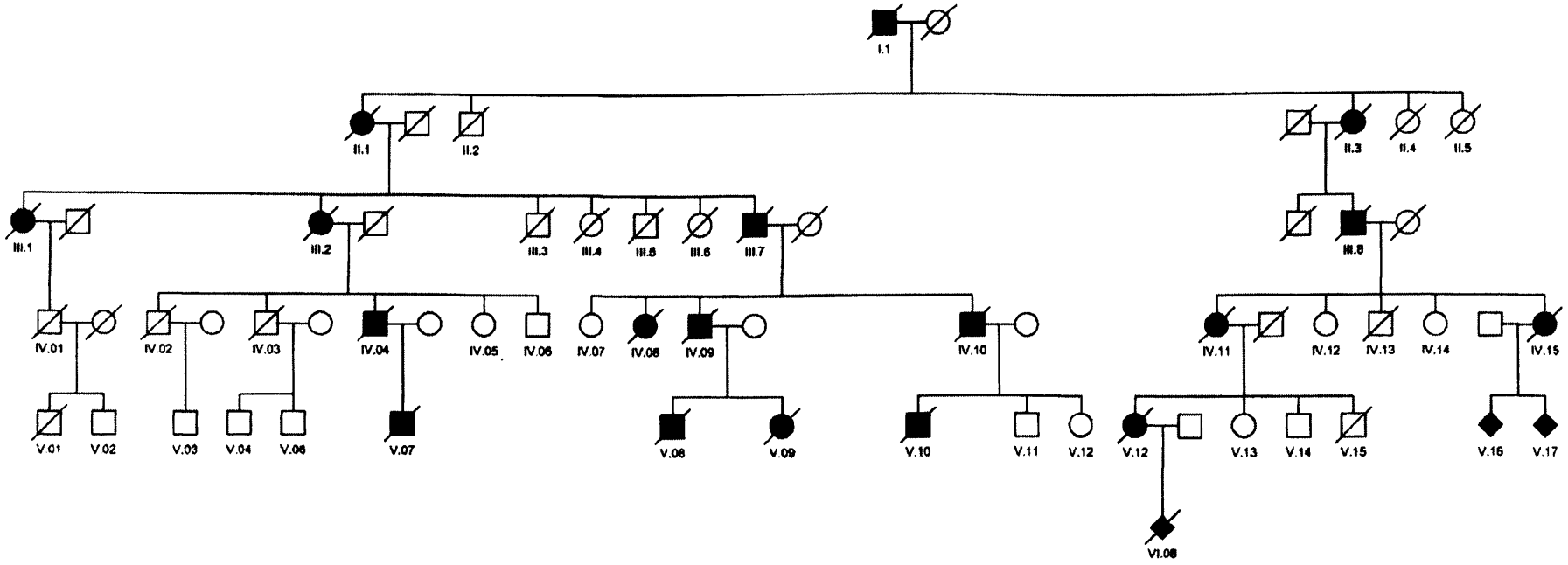
4.2.1.5 Neuropathology

The brain weight of case V.10 was 963g, and the weight of the brainstem and the cerebellum 174g. The leptomeninges were thickened and the large cerebral arteries showed a few atherosclerotic plaques with up to 50% narrowing of the lumina. In case VI.08 the brain weighed 1051g and the cerebellum with the brainstem 162g. The leptomeninges were normal, but there were occasional small atherosclerotic plaques in the large cerebral vessels. The remaining neuropathology was identical for both cases and shall be described together. The cranial nerves were normal. There was severe diffuse tissue loss affecting all lobes, but more pronounced in the frontotemporal region: the gyri here were considerably narrowed and the intervening sulci widened. The lateral ventricles were greatly enlarged with rounding of the angle. Both the substantia nigra and the locus coeruleus were paler than usual. The histological sections showed many neurofibrillary tangles, neuropil threads and neuritic plaques throughout the neocortex (see Figure 4.3). In places there was superficial status spongiosus accompanied by astrocytosis indicating neuronal loss. The hippocampus showed neuronal loss from the CA1 area, many neurofibrillary tangles, senile plaques, granulovacuoles and Hirano bodies (see Figure 4.4). The nucleus basalis of Meynert was depopulated and many of the surviving neurons contained neurofibrillary tangles (see Figure 4.5). A couple of neurofibrillary tangles were also seen in the caudate nucleus, in the lentiform nucleus, in

the claustrum and in the subthalamic nucleus and several more were noted in the thalamus. The substantia nigra and the locus coeruleus showed neuronal loss, extraneuronal pigment and neurofibrillary tangles. These were also seen in the raphe nuclei and in the periaqueductal grey matter. There was slight focal Purkinje cells loss in the cerebellum with accompanying mild to moderate astrocytosis of Bergmann glia. The walls of the small and medium-sized cerebral and leptomeningeal blood vessels were thickened, and often appeared homogeneously eosinophilic with occasional double barrelling, particularly in case V.10. The enlarged perivascular space contained mononuclear cells including lymphocytes and pigment-bearing macrophages. Immunohistochemistry for β-amyloid showed extensive deposition in the grey matter (see Figure 4.6) as well as in the leptomeningeal and parenchymatous blood vessels. In the cerebellum the β-amyloid deposition was more severe in the blood vessels than in the parenchyma. Tau immunostaining confirmed all the cytoskeletal abnormalities, while reaction for α-synuclein was negative in VI.08, but revealed several Lewy bodies in the sustantia nigra, the locus coeruleus, the frontal and insular cortices, and in the parahippocampal gyrus of case V.10. GFAP highlighted astrocytosis in the cortex, subcortical white matter and, to a lesser extent, in the deep grey structures.

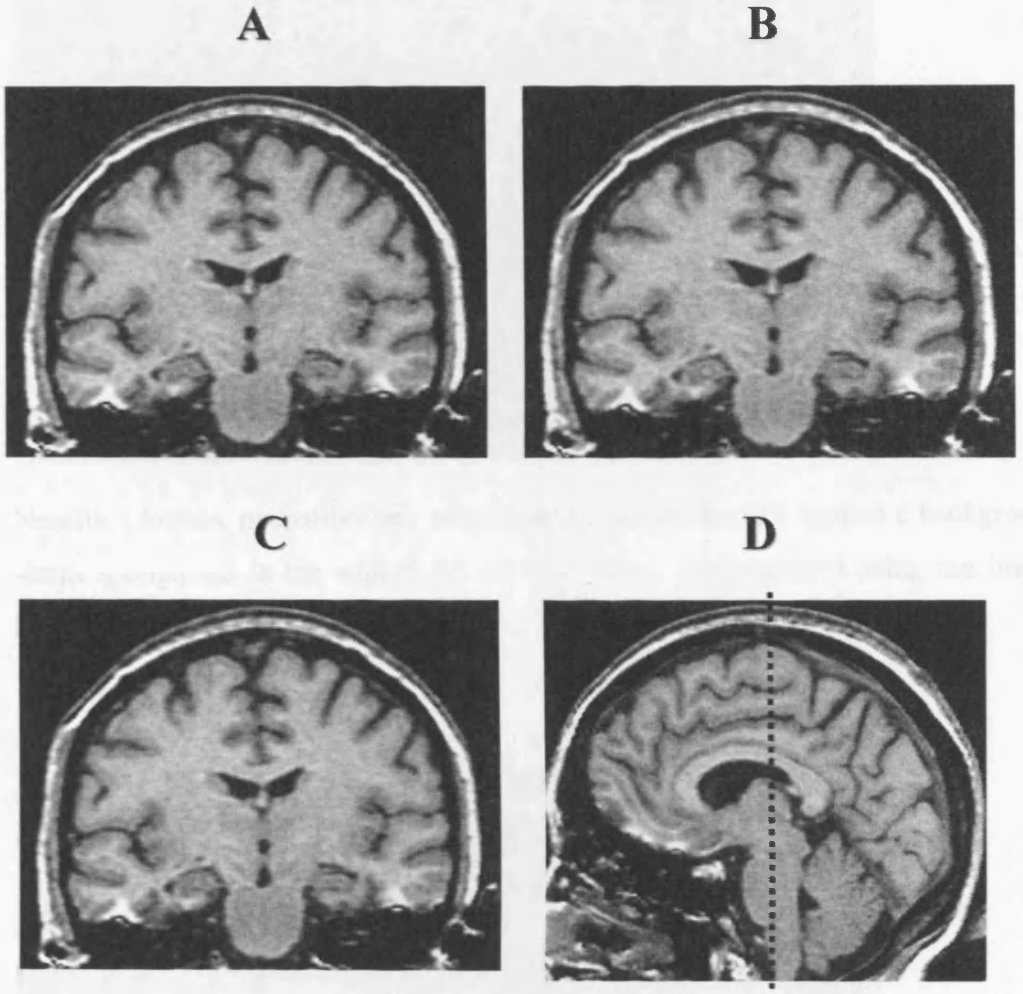
In conclusion both cases showed severe Alzheimer's disease fulfilling the CERAD as well as the more recent National Institute of Aging and Reagan Institute criteria for the diagnosis of definite Alzheimer's disease (Hyman & Trojanowski 1997) with extensive deposition of β-amyloid both in the grey matter and in the cerebral blood vessels.

Figure 4.1 Family tree of family 105/160



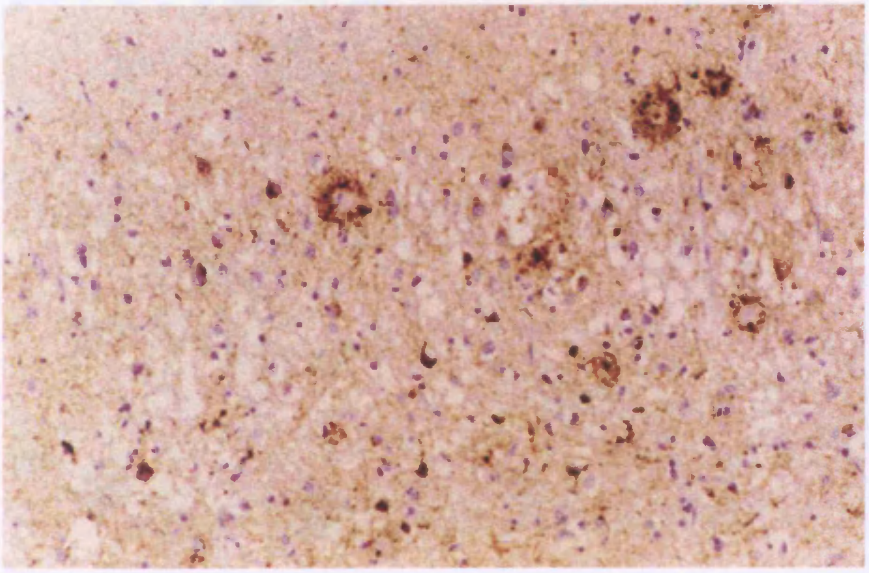
Solid symbols are affected family members, open symbols are unaffected members, squares are males and circles are females. Diagonal line represents deceased members. For reasons of anonymity the current generation is represented by diamonds, and their "at risk" offspring are not shown.

Figure 4.2 Brain MRI of subject 105/160.V.17



Images A, B and C are registered coronal T_1 weighted MR brain images taken respectively at presentation, 13 and 22 months later. D = sagital T_1 weighted MR brain image demonstrating the level at which coronal slices A-C have been taken.

Figure 4.3 Neuropathology of 105/160.VI.08 (parietal cortex)



Neuritic plaques, neurofibrillary tangles and neuropil threads against a background of status spongiosus in the superficial parietal cortex demonstrated using tau immuno-histochemistry (original magnification x 251).

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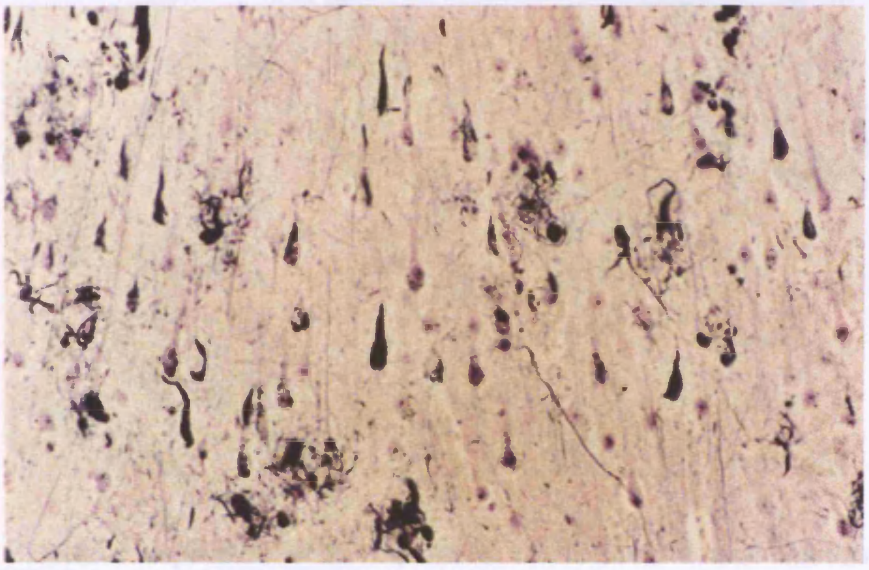
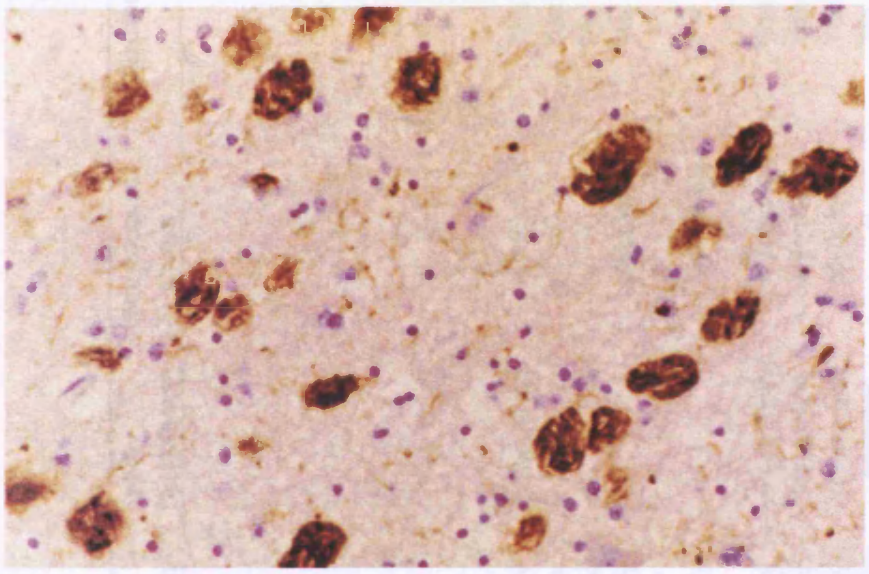
Figure 4.4 Neuropathology of 105/160.VI.08 (hippocampus)



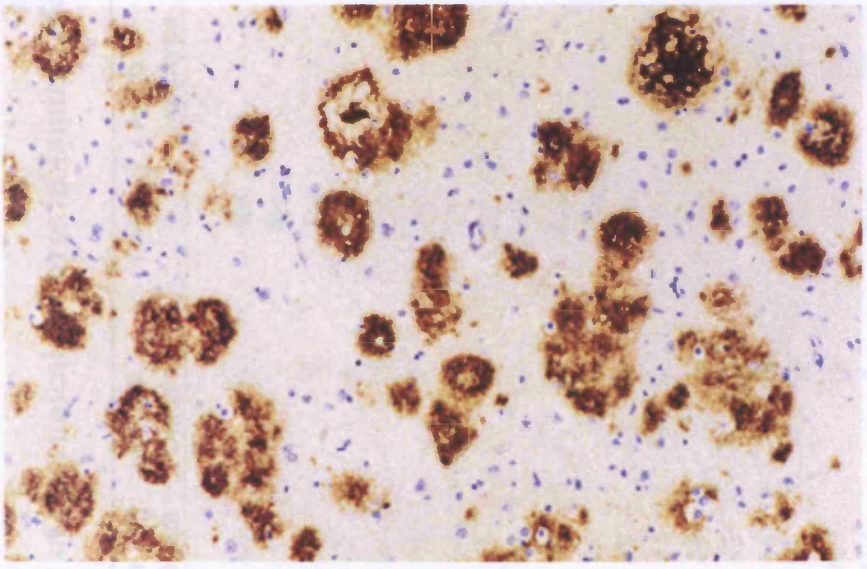
Neurofibrillary tangles and neuritic plaques in the hippocampus demonstrated with a modified Bielschowsky silver impregnation (original magnification x 251).

Figure 4.5 Neuropathology of 105/160.VI.08 (nucleus basalis)



Neurofibrillary tangles in the nucleus basalis of Meynert demonstrated using tau immuno-histochemistry (original magnification x 465).

Figure 4.6 Neuropathology of 105/160.VI.08 (cerebral cortex)



Extensive deposition of β amyloid in the cerebral cortex demonstrated using βA4 immunostaining (original magnification x 251).

Table 4.1 Summary of the clinical features of family 105/160

Subject	Age (yr) at onset	Age (yr) at Death	Illness Duration (yr)	Autopsy	ApoE genotype	NINCDS-ADRDA	First Symptom	Other Symptoms	Neurological Features	Seizures
II.1	-	46	-	No	-	-	-	-	-	-
II.3	-	-	-	No	-	-	-	-	-	-
III.1	37	40	3	No	-	-	-	-	-	-
III.2	37	42	5	No	-	-	-	-	-	-
III.7	39	47	8	No	-	-	-	-	-	-
III.8	38	44	6	No	-	-	-	-	-	-
IV.04	38	47	9	No	-	-	-	-	-	-
IV.08	37	45	8	No	-	-	-	-	-	-
IV.09	37	45	8	Yes	-	Probable	Memory	Wandering	Myoclonus	Yes
IV.10	38	48	10	No	-	Probable	Memory	-	0	Yes
IV.11	36	41	5	Yes	-	Definite	Depression	-	0	No
IV.15	38	45	7	Yes	34	Definite	Memory	-	Myoclonus	Yes
V.07	-	41	-	-	-	-	-	-	-	-
V.08	40	44	4	No	-	Probable	Memory	-	Myoclonus	No
V.09	36	43	7	-	-	-	-	-	-	-
V.10	39	54	15	Yes	33	Definite	Memory	Personality	Myoclonus	Yes
V.12	40	45	5	No	-	Probable	Memory	Personality	Myoclonus	Yes
V.16	33	N/A	12	N/A	44	Probable	Memory	Personality	Myoclonus	Yes
V.17	36	N/A	5	N/A	33	Probable	Repetitive	Memory	0	No
VI.08	36	43	7	Yes	33	Definite	Slowness	Memory	Myoclonus	Yes

N/A = not applicable, 0 = absent, '-' = unknown

Table 4.2 Neuropsychology data for family 105/160

	IV.15			V.16				V.17		VI.08
Assessment	**1st**	**2nd**	**3rd**	**1st**	**2nd**	**3rd**	**4th**	**1st**	**2nd**	**1st**
Time (months)	*0*	*10*	*21*	*0*	*12*	*23*	*48*	*0*	*24*	*0*
WAIS-R VIQ	70	66	<54	92	84	82	81	110	103	64
WAIS-R PIQ	<56	<56	<56	76	73	65	57	116	86	59
Scaled Scores										
Digit Span	1	4	0	10	7	8	4	14	12	2
Vocabulary	10	8	7	9	8	8	8	11	10	7
Arithmetic	5	5	2	8	8	5	4	10	9	4
Similarities	8	5	3	9	7	7	10	12	12	4
Picture Completion	6	3	2	6	8	5	4	15	9	5
Picture Arrangement	NT	NT	NT	6	5	5	2	12	9	3
Block Design	0	0	0	6	4	3	2	9	7	0
Digit Symbol	0	0	0	NT	NT	NT	NT	NT	NT	NT
Object Assembly	2	2	0	NT	NT	NT	NT	NT	NT	NT
RMT words	NT	NT	NT	<5th	<5th	<5th	NT	<5th	<5th	<5th*
RMT faces	NT	NT	NT	25th	25th	10th	<5th**	10th	25th	<5th*
GNT	NT	NT	NT	75-90th	NT	90th	75-90th	95th	95th	<1st
NART	NT	NT	NT	>75th	>75th	>75th	>75th	>75th	NT	10-25th
GDST	NT	NT	NT	NT	>75th	>75th	50th	>75th	>75th	<1st
GDAT	NT	NT	NT	NT	5th	<1st	<1st	>75th	75th	<1st
VOSP										
Silhouettes (%)	NT	NT	NT	NT	25th	10-25th	5-10th	>75th	>75th	NT
Fragmented Letters***	NT	NT	NT	Pass	NT	NT	NT	NT	NT	Fail
Cube Analysis***	NT	NT	NT	Pass	Pass	Fail	NT	Pass	Pass	NT

NT = not tested
WAIS-R = Wechsler Adult Intelligence Scale Revised (Wechsler, 1981)
VIQ = verbal IQ; PIQ = performance IQ;
RMT= Recognition Memory Test (Warrington 1986)
*/** = Shortened/Shortened and Easy version of the RMT
GNT = Graded Naming Test (McKenna, 1986)
NART = National Adult Reading Test (Nelson, 1991)
GDST = Graded Difficulty Spelling Test (Baxter, 1994)
GDAT = Graded Difficulty Arithmetic Test (Jackson, 1986);
VOSP = Visual Perceptual and Space Perception Battery
(Warrington, 1991); *** 5% cutoff used to determine pass/fail

4.2.2 PSEN1 L153V mutation

The *PSEN1* L153V mutation was first reported in a small French pedigree without neuropathological confirmation (Raux *et al.* 2000). This mutation is located in exon 5 of *PSEN1*. The clinical and neuropathological features of family 177, that also carries the *PSEN1* L153V mutation, are reported here. Figure 4.7 shows the family tree of this small pedigree. Patients III.2 and III.3 were assessed clinically.

4.2.2.1 Family overview

The clinical findings are summarised in Table 4.3. Mean age at onset of symptoms was 35.3 years (95% CI: 34.6-36.0 years), mean age at death was 44.0 years (95% CI: 39.1-48.9 years) and mean duration of illness was 8.3 years (95% CI: 4.7-11.9 years). Patients III.2 and III.3 were assessed clinically.

4.2.2.2 Case reports and neuropsychology

II.5 Age at onset 35 years; age at death 42 years; duration 7 years. This right-handed factory worker presented at the age of 40 years with a five year history of progressive memory problems. Neurological examination was normal. Baseline dementia screening blood tests were normal; the Wassermann reaction was negative. A ventriculogram showed good filling of the ventricular system without evidence of a space occupying lesion. A diagnosis of presenile dementia was established. At the age of 41 years he was admitted to a psychiatric hospital, he was moderately demented and required nursing care. He evaded answering direct questions. He appeared happy and cheerful but was totally disorientated. His memory was impaired and he had a digit span of three figures forwards. He was unable to perform simple calculations. He died of bronchopneumonia two years later. The death certificate recorded bronchopneumonia and presenile dementia as the causes of death. Neuropathological examination was not performed although Alzheimer's disease was felt to be the likely diagnosis.

III.2. Age at onset 35 years; age at death 41 years; duration six years. This man worked as a draughtsman. He was referred at the age of 38 years with a three year history of personality change, progressive memory impairment and difficulties at work. His past medical history was unremarkable. His wife noted a personality change in that he had become quieter and less outgoing. He subsequently had

progressive memory failure and difficulty with his job. At the age of 37 years his employer referred him to their occupational health department because of poor work performance. He was aware of some of his cognitive difficulties, in particular with regards to memory. One year later he was referred to a neurologist who documented evidence of cognitive impairment. On formal testing (see Table 4.4) he obtained a full-scale IQ of 71 on the Revised Wechsler Adult Intelligence Scale (WAIS-R) (Wechsler 1981), which represented a moderate intellectual deterioration from his premorbid IQ estimate of 106 on the National Adult Reading Test (NART) (Nelson & Willison 1991). Deficits were also recorded in memory, spelling, writing, and reading. On neurological examination he was found to be mildly dysarthric, and to have asymmetric impairment of co-ordination in his right hand. He had a brisk pout and positive grasp reflex. Extensive investigations were normal including biochemical and haematological profiles, syphilis serology, serum vitamin B_{12}, red cell folate and thyroid function tests. A CT brain scan showed some minor degenerative changes and the EEG showed bilateral slowing of the background activity consistent with a diagnosis of Alzheimer's disease. Examination of his CSF was normal, as was an EMG. He progressively deteriorated and died without neuropathological examination.

III.3. Age at onset 36 years; age at death 49 years; duration 13 years. This left-handed bookkeeper presented at the age of 37 years with a one year history of memory impairment and anxiety symptoms related to her family history of dementia. She complained of difficulty with mental arithmetic and impaired episodic memory. Her past medical history was unremarkable. There was no history of head injury, epilepsy, or psychiatric illness. She did not drink alcohol and was a non-smoker. She underwent neuropsychometric assessment at this time (see Table 4.4) and obtained average Verbal and Performance IQ measures, which were in keeping with her optimal level of functioning as estimated using the NART. The recognition memory test (RMT) (Warrington 1984) revealed a mild global weakness in her memory functions. Nominal skills, as assessed using a naming by definition task, were preserved and in keeping with her expressive vocabulary. She performed flawlessly on the Unusual Views test (Warrington & James 1988) and on a subtest (Fragmented Letters) of the Visual Object and Space Perception Battery (VOSP) (Warrington & James 1991), which were used to obtain a measure of perceptual skills. An unenhanced brain CT was reported as being normal. There was felt to be insufficient evidence of significant cognitive impairment. Over the next 18 months she was

downgraded at work, changed jobs and started work as a domestic cleaner and reported further deterioration of her memory. She also entered into a new marriage, but this was short-lived and her husband left her suddenly. She was having difficulties remembering lists, telephone numbers and appointments. A second CT brain scan showed cortical atrophy, but she was lost to follow up for three years. She was reassessed aged 41 years. By then she was having significant problems with episodic memory; in particular she could not remember the events of the previous day and the purpose of a visit outside the home. There were no difficulties with language or topographical memory. She no longer watched television or read books, as she could not retain what had gone before. She continued to be self caring, although she was not as fastidious as she had been. Her culinary skills had declined and she could no longer follow a recipe. She was very repetitive in conversation and often disorientated. Mini-mental state examination was 12/30. General examination was normal. Neurological examination revealed normal gait. There was no amyotrophy, fasciculation or myoclonus. Tone was normal and there were no extrapyramidal signs. Tendon reflexes were symmetrical and plantars flexor. There was evidence of dyspraxia, with impaired copying of gestures and limb substitution. Cranial nerve examination showed a visual acuity of 6/6 bilaterally, with normal fundoscopy. There was a full range of ocular movements although pursuit movement was broken. The jaw jerk was slightly brisk. The remaining cranial nerves were normal. Neuropsychometry was repeated (see Table 4.4) and she obtained a borderline defective Verbal IQ and a defective Performance IQ. Her memory functions had gravely deteriorated; performance on the RMT for words was at chance and on the RMT for faces below the 1st percentile. Nominal skills were relatively well preserved on the Oldfield Picture Naming Test (Oldfield & Wingfield 1965). Visuoperceptual functions had deteriorated: she scored below the 5th percentile on the Silhouettes subtest of the VOSP. Frontal executive functions as assessed on the Weigl Sorting Test were normal (Weigl 1948). She performed poorly on a letter cancellation task. Dementia screening blood tests and CSF examination were normal. Brain MRI (see Figure 4.8) showed marked atrophy, particularly involving the parietal lobes bilaterally. The posterior parts of the frontal lobes were also affected, especially the region of the paracentral lobules on each side. The cerebellum appeared mildly atrophic. The temporal lobes were relatively spared, although careful visual inspection of the medial temporal structures suggested that the amygdalae might have been slightly atrophied. Little or

no white matter disease beyond the immediate periventricular zone was seen. The ventricles were mildly enlarged particularly in the posterior parts of the bodies and trigones. The following year she entered long term nursing care and was noted to have developed myoclonus, which was treated with carbamazepine. She died of bronchopneumonia at the age of 49 years and underwent neuropathological examination.

4.2.2.3 Molecular Genetics

In this small pedigree DNA was only available for patient III.3. The L153V mutation was demonstrated in patient III.3 and was absent from 100 unrelated normal controls from the same ethnic background.

4.2.2.4 Neuropathology

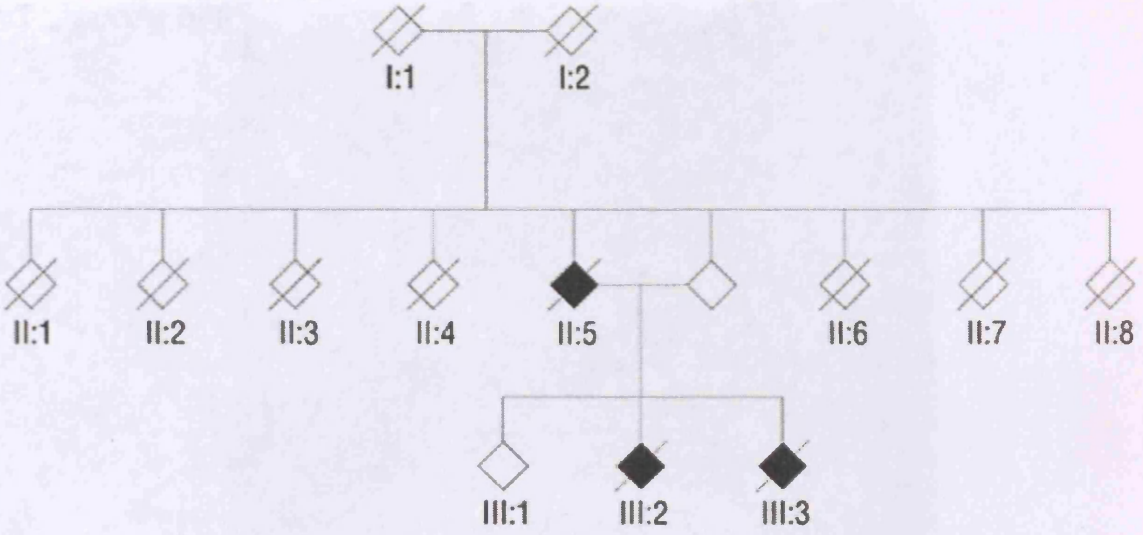
Only case III.3 was examined. The brain weighed 930g, the brainstem and cerebellum 128g. The leptomeninges were thickened and the large cerebral blood vessels showed an occasional atherosclerotic plaque. There was severe generalised atrophy affecting all lobes of the brain. This was confirmed on coronal slicing: the lateral ventricles were greatly enlarged with rounding of the angle, the gyri were narrowed, and the intervening sulci widened.

Histological examination showed features of severe Alzheimer's disease: neurofibrillary tangles, neuritic plaques and neuropil threads were abundant in the hippocampus and the cerebral cortex (see Figure 4.9). In the hippocampus there was severe neuronal loss accompanied by astrocytosis. Superficial status spongiosus and astrocytosis were noted focally in the cerebral cortex, indicating severe neuronal loss. Neurofibrillary tangles occurred also in the deep grey matter, including the nucleus basalis of Meynert, the lentiform nucleus, substantia nigra, locus coeruleus, periaqueductal grey matter and the raphe nuclei.

Immunohistochemistry for α-synuclein revealed an occasional Lewy body in the transentorhinal, the cingulate, the insular and parietal cortices, as well as in the nucleus basalis of Meynert (see Figure 4.10). There was extensive deposition of β-amyloid both in the cerebral parenchyma and in the blood vessels (see Figure 4.11).

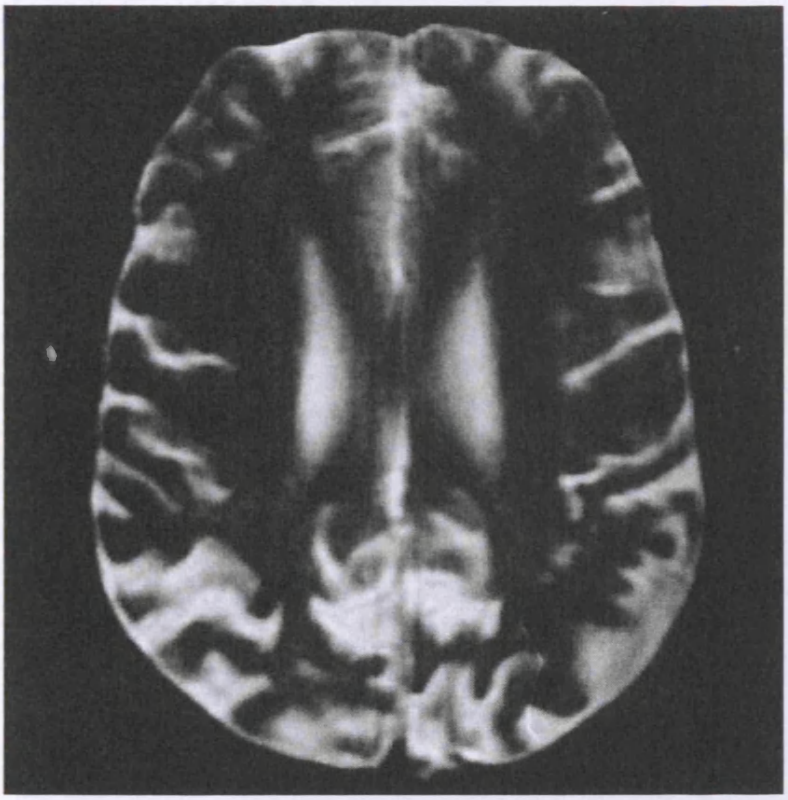
The neuropathological diagnosis was severe Alzheimer's disease with amyloid angiopathy with additional Lewy body pathology noted.

Figure 4.7 Family tree of family 177



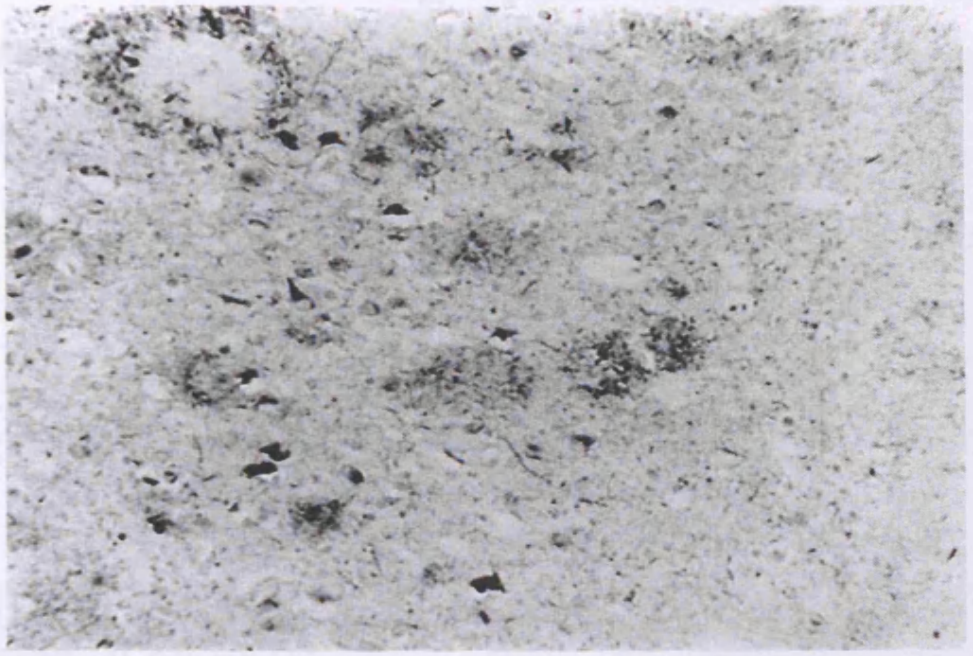
To preserve confidentiality this pedigree has been anonymised.

Figure 4.8 MRI of patient 177.III.3



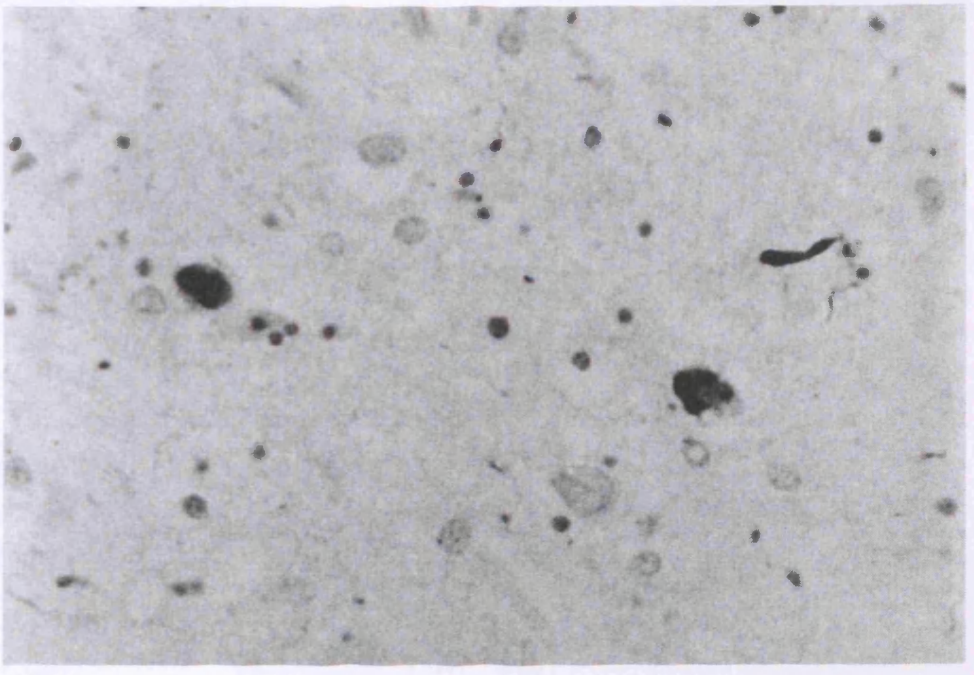
T2 weighted axial MRI showing biparietal atrophy

Figure 4.9 Neuropathology of patient 177.III.3 (temporal cortex)



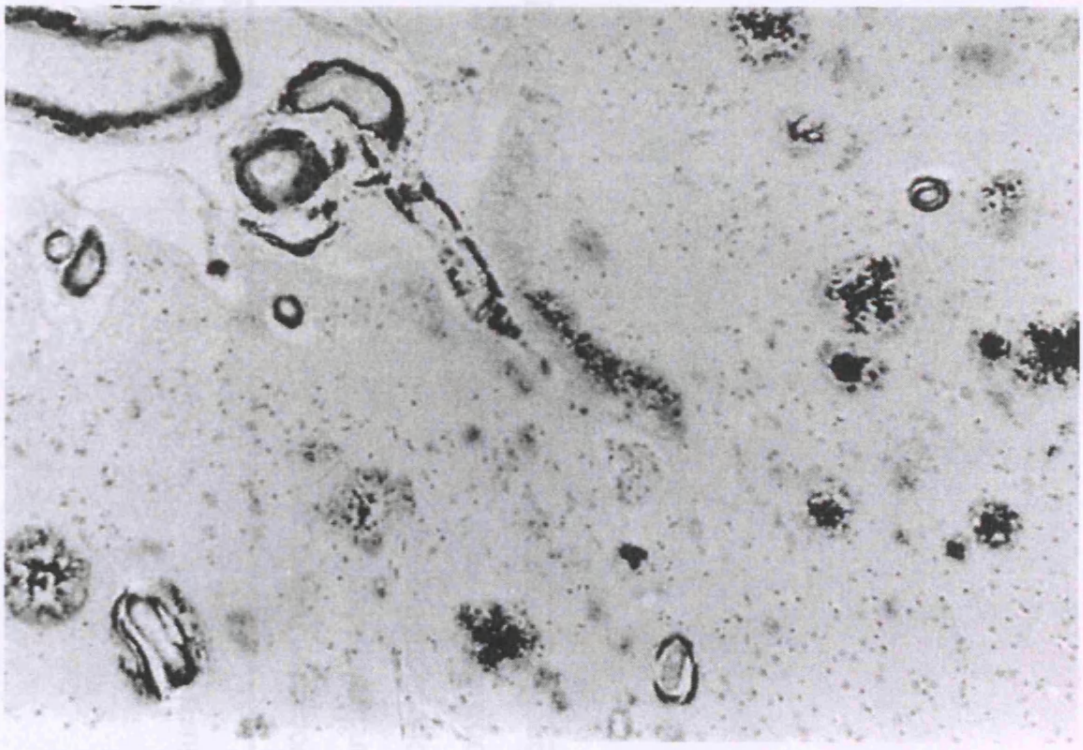
Senile plaques, neurofibrillary tangles, and neuropil threads demonstrated using tau protein immunostaining (original magnification x 70).

Figure 4.10 Neuropathology of patient 177.III.3 (amygdala)



Lewy bodies using α-synuclein immunostaining (original magnification x 120).

Figure 4.11 Neuropathology of patient 177.III.3 (cerebral cortex)



Immunostaining of β-amyloid in cerebral parenchyma, lepto meningeal and cerebral blood vessels (original magnification x 30).

Table 4.3 Summary of clinical features of family 177

Subject	Age (y) at onset	Age (y) at death	Illness Duration (y)	First Symptom	Cause of death	Psychiatric Features	Neurological Features
I.1	NA	65	NA	Unaffected	Myocardial Infarction		NA
I.2	NA	73	NA	Unaffected	Carcinomatosis		NA
II.5	35	42	7	Memory	Bronchopneumonia	-	-
III.2	35	41	6	Memory	Bronchopneumonia	Personality change	-
III.3	36	49	12	Memory	Bronchopneumonia	-	Myoclonus

Table 4.4 Neuropsychology results of patient 177.III.3.

Test Occasion	1	2
WAIS-R FIQ	98	72
WAIS-R VIQ	99	76
WAIS-R PIQ	97	65
NART	102	-
RMT Words	43/50	27/50
RMT Faces	41/50	33/50
Naming by definition	14/15	-
Oldfield Picture Naming	-	22/30
Unusual Views	20/20	-
Fragmented Letters	20/20	-
Silhouettes	-	15/30

"-" signifies not tested, remaining abbreviations as in text

4.2.3 PSEN1 E280G mutation

The E280G *PSEN1* mutation was first demonstrated in two DRG families, F168 and F183, which are both from Ireland (Clark *et al.* 1995e). The mutation is one of two at codon 280 and one of 23 in exon 8 of *PSEN1*. It has also been demonstrated in F102 a large Irish family in which there have been nine affected members in three generations. The clinical care for this family has been with Professor Michael Hutchinson, consultant neurologist, St Vincent's University Hospital, Dublin. Several "at risk" family members come to London annually to take part in the DRG longitudinal study. From the limited historical data that are available the typical phenotype has been one of dementia and myoclonus with onset early in the fifth decade. The clinical and neuropathological features of three affected individuals from family 102 with a rather different phenotype are reported here. Figure 4.12 shows the family tree of family 102.

4.2.3.1 Family overview

The clinical findings are summarised in Table 4.5. Mean age at onset of symptoms was 40.0 years (range 38.0-43.0 years), mean age at death was 50.0 years (range: 48.0-52.0 years) and mean duration of illness was 11.0 years (range 10.0-12.0 years). Patients III.2 and III.3 were assessed clinically in Dublin, whereas patient III.9 was assessed clinically in Dublin and for research purposes in London.

4.2.3.2 Case reports and neuropsychology

III: 9. Age at onset 43 years; current duration 5 years. This 48 year old man presented with a five-year history of stiffness and weakness in his legs. The onset of this symptom was insidious and the progression gradual. He had had to stop work as a municipal gardener because of his progressive gait difficulties. He also complained of urinary urgency and frequency. Against a background of marked anxiety, he had also noticed some minor memory problems. Although the family history is censored by FAD his only identifiable vascular risk factor was a 20 pack-year history of cigarette smoking. General examination revealed that he was normotensive. He scored 23/30 on the MMSE. On the CAMCOG neuropsychological battery he obtained a score of 71/107 (normal >80), indicating mild dementia (Roth *et al.* 1986). More detailed neuropsychological assessment was hampered by anxiety and limited schooling. He

had particular difficulty with registration, recall and language expression. Apart from a brisk jaw-jerk, cranial nerve examination was normal. Tone and power were normal in his upper-limbs. There was evidence of mild finger-nose incoordination and upper limb reflexes were brisk. He had a spastic paraparesis with MRC grade 4 weakness bilaterally in a pyramidal distribution. He had bilateral ankle clonus and extensor plantar responses. He had no sensory impairment. He had a spastic-ataxic gait. Investigations included normal full blood count, erythrocyte sedimentation rate, electrolytes, liver enzymes and thyroid function tests. Vitamin B_{12} level was normal. Very long chain fatty acid levels were normal. Fasting lipid profile was normal. Chest X-ray and ECG were normal. Cranial MRI was abnormal (see Figure 4.13). There were minor changes of atrophy but no other abnormality on T1-weighted images. On T2-weighted and FLAIR images, there were extensive periventricular areas of high signal intensity. Foci of high signal intensity were also noted in the deep white matter bilaterally and in the pons and medulla. There were no abnormalities on whole spine MRI. Routine analysis of CSF was normal and oligoclonal bands were absent. Visual evoked potentials were normal. Antinuclear antibody (ANA) was mildly positive, but tests for antibodies to anti-double-stranded DNA and extractable nuclear antigens were negative. Antineutrophil cytoplasmic antibody (ANCA) was negative. Doppler ultrasound of carotid and vertebral arteries revealed no abnormalities.

III.3. Age at onset 40 years; age at death 52 years; duration 12 years. This patient presented at the age of 43 years with moderate dementia. His initial complaint had been one of deteriorating memory. In addition to obvious abnormalities of higher mental function, neurological examination revealed prominent myoclonus. Spastic paraparesis was not reported. His cranial MRI had evidence of white matter abnormalities.

III:2. Age at onset 38 years; age at death 48 years; duration ten years. This patient presented at age 40 years with cognitive impairment and two years later developed gait difficulties. She also complained of diplopia. On examination she had evidence of an internuclear ophthalmoplegia and a spastic-ataxic quadriparesis. A brain CT was normal, but unfortunately cranial MRI was not performed. Because of her somewhat unusual presentation at a time when the family diagnosis was unconfirmed a brain-biopsy was performed. She died within ten years of onset of cognitive impairment.

4.2.3.3 Molecular Genetics

Patient III.9 underwent diagnostic genotyping. The complete open reading frame of exon 8 of the *PSEN1* was sequenced and the E280G mutation demonstrated. Presence of the mutation was confirmed using allele specific oligonucleotide hybridisation.

4.2.3.4 Neuropathology

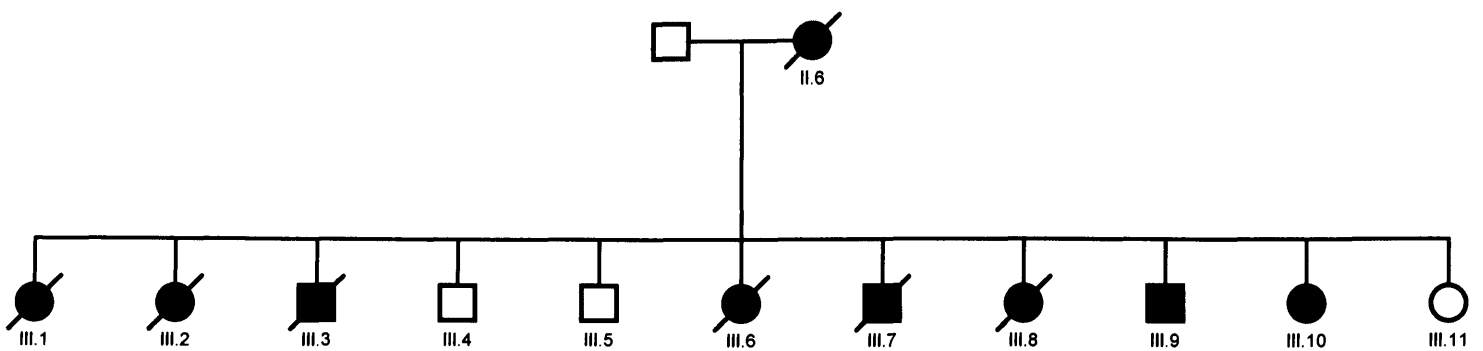
Patient III.2 had previously undergone brain biopsy. This was re-evaluated by Professor Michael Farrell, Department of Neuropathology, Beaumont Hospital, Dublin. It revealed numerous, large, non-cored, "cotton-wool" plaques (see Figure 4.14). Cerebral amyloid angiopathy due to Aβ deposition was widespread, affecting both cortical and meningeal vessels. Neurofibrillary tangles and neuropil threads were ubiquitous, although tau-positive neurites were sparse. Although significant deposition of Aβ was observed, no neuritic pathology was associated with these lesions.

Table 4.5 Summary of clinical features of family 102

Subject	Age (y) at onset	Age (y) at death	Illness Duration (y)	First Symptom	Other Symptom	Imaging Features	Neuropathology
III.2	38	48	10	Memory	SP, INO	CT normal	CWP on Biopsy
III.3	40	52	12	Memory	Myoclonus, No SP	MRI: WM abnormalities	-
III.9	43	Alive	5+	SP	Memory, Anxiety	MRI: WM abnormalities	-

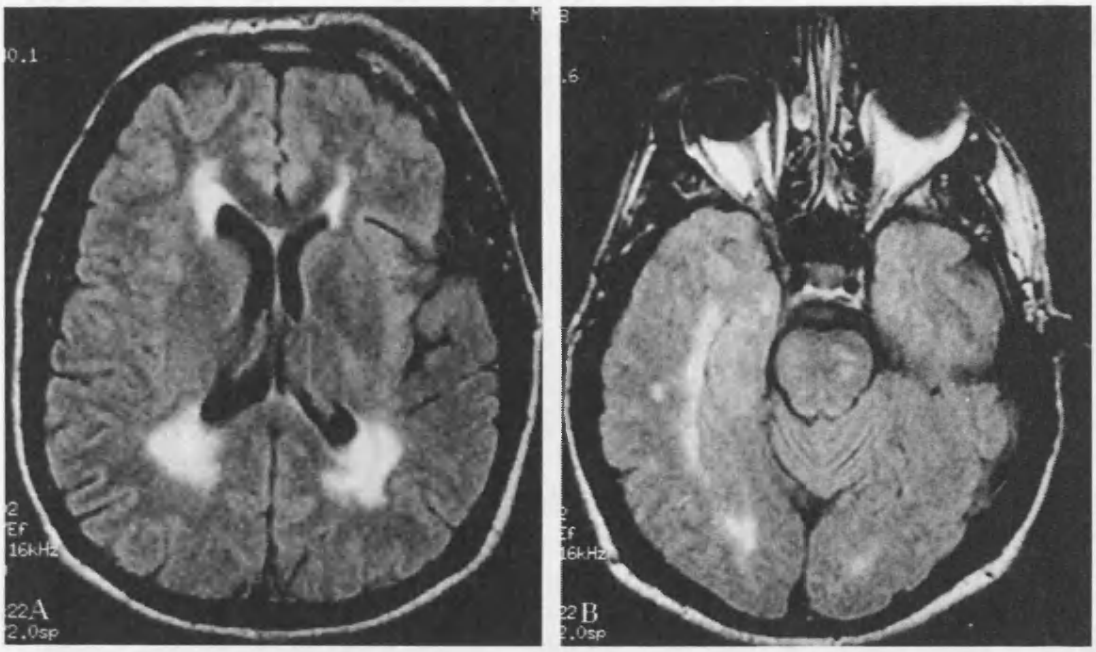
SP = Spastic Paraparesis, INO = Internuclear Ophthalmoplegia, CWP = "Cotton Wool" Plaques, WM = White Matter

Figure 4.12 Family tree of family 102



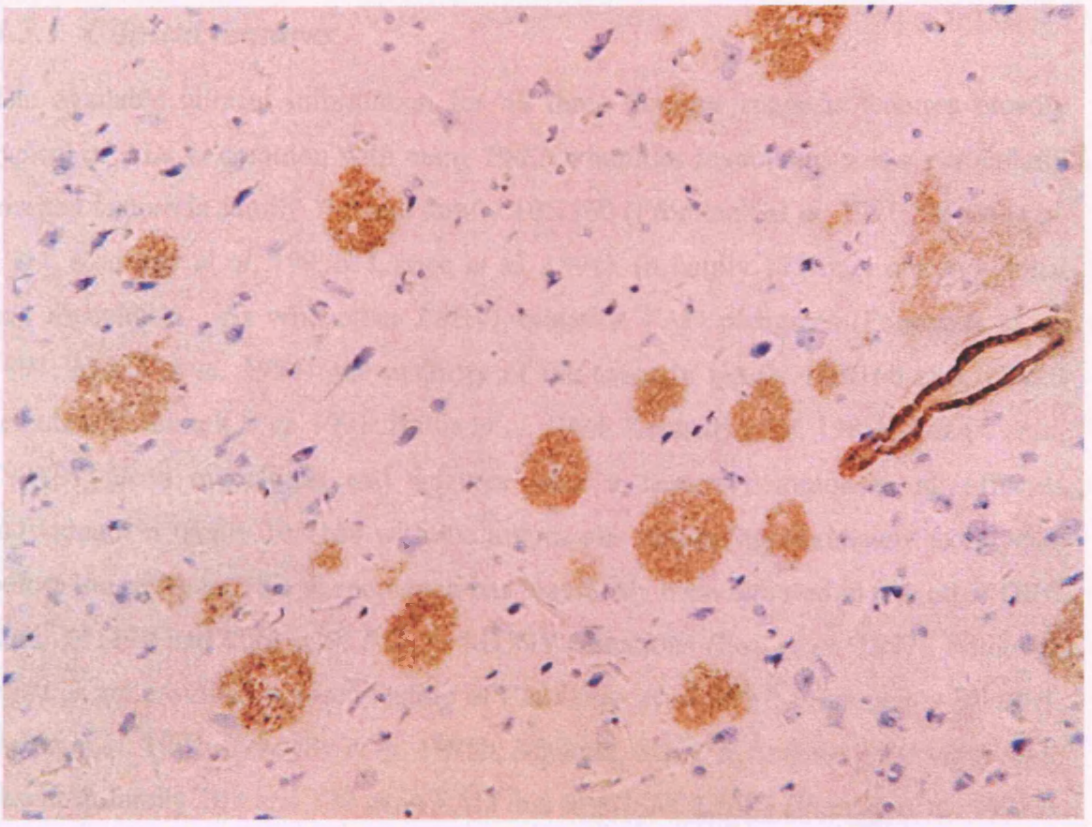
Solid symbols are affected family members, open symbols are unaffected members, squares are males and circles are females. For reasons of anonymity the current generation is represented by diamonds, and their "at risk" offspring are not shown.

Figure 4.13Cranial MRI of patient 102.III.9



MRI (FLAIR sequence) shows areas of periventricular high signal intensity. There are additional discrete foci of high signal intensity in the deep white matter bilaterally. Foci of high signal intensity are also present within the brainstem.

Figure 4.14 Neuropathology of patient 102.III.2



Section of temporal neocortex immunostained with βA4 antibody (DAKO) to show cotton wool plaques together with immunopositivity of a parenchymal vessel (original magnification x 125)

4.3 Discussion

4.3.1 Clinical features

The available clinical information for all three families suggests features broadly typical of AD. In common with many *PSEN1* families myoclonus was a particularly marked feature in family 102 and family 105/160 (Frommelt *et al.* 1991; Haltia *et al.* 1994; Kennedy *et al.* 1995b; Lampe *et al.* 1994). In family 177 only one individual had myoclonus. As with other *PSEN1* mutation FAD pedigrees (Frommelt *et al.* 1991; Haltia *et al.* 1994) the majority of the cases in family 105/160 experienced seizures (Frommelt *et al.* 1991; Haltia *et al.* 1994; Kennedy *et al.* 1995b; Lampe *et al.* 1994). Indeed myoclonus and seizures were a feature common to all affected individuals in family 105/160 with the myoclonus starting approximately three years before the seizures. This phenomenon has previously been reported in two other DRG families (148 and 206) with *PSEN1* M139V mutations (Fox *et al.* 1997). Although seizures are a well recognised feature of FAD (Fox *et al.* 1997; Frommelt *et al.* 1991; Haltia *et al.* 1994; Kennedy *et al.* 1995b; Mayeux, Stern, & Spanton 1985) they were absent in family 102 and 177 as in FAD due to *PSEN1* L250S (Harvey *et al.* 1998a). Myoclonus and seizures have not been reported in the French L153V family (Raux *et al.* 2000).

The main phenotypic difference between these three families and the majority of *PSEN1* kindreds appears to be a very early AAO: 40 years in family 102, 37.4 years in family 105/160, and 35.3 years in family 177. In a review of 38 published pedigrees with 22 different PSEN1 mutations, only seven pedigrees had a mean AAO in the fourth decade of life (Cruts, Hendriks, & Van Broeckhoven 1996). There does not seem to be a clear correlation between AAO and site of the mutation. Even for the TM2 mutation cluster, where the thirteen known mutations are in close juxtaposition, there is a 23-year range in AAO. Data for this cluster are available for 25 of the 29 published pedigrees (see Table 4.6): eleven had a mean AAO in the fourth decade of life; fourteen in the fifth decade; and for one family the mean AAO was in the sixth decade of life. The nature of individual mutations may account for a degree of this variation as illustrated by the two mutations at codon 143: a non-conservative change (I143T) has a mean AAO of 35 years, whereas a semi-conservative change (I143F)

has a mean AAO of 55 years. However, this would not account for the variation seen in AAO for the same mutation and even within some families.

For APP families the dose of *APOE ε4* alleles has been shown to reduce the AAO (Alzheimer's Disease Collaborative Group 1993; Mullan *et al.* 1993). Because this effect has been shown to be absent in multiple *PSEN1* families (Lendon *et al.* 1997; Van Broeckhoven *et al.* 1994a) further unidentified genetic factors have been proposed for these families (Axelman, Basun, & Lannfelt 1998; Fox *et al.* 1997). However, in family 105/160 *APOE* may modulate the disease. Patient 105/160.V.16 (*APOE ε4/ε4*) had an age at onset of 33 years which was more than one standard deviation below the mean age of onset for those of known APOE genotype (36.4±2.3 years, mean±SD), and more than two standard deviations below the mean age of onset for the pedigree as a whole (37.4±1.7 years, mean±SD).

Spastic paraparesis was present in two family members of family 102, but absent from all family members of families 105/160 and 177. Patient 102.III.2 had presented with dementia in association with a spastic tetraparesis and an internuclear ophthalmoplegia. The spastic paraparesis phenotype is absent from the two other Irish DRG families carrying this same E280G mutation (Clark *et al.* 1995e). A second reported mutation at codon 280, found in a large Colombian family with the *PSEN1* E280A mutation is not associated with spastic paraparesis (Lopera *et al.* 1997). The FAD spastic paraparesis phenotype is associated with "cotton-wool" plaques first described in a large Finnish pedigree with a genomic deletion of exon 9 (*PSEN1* Δ9), though not all *PSEN1* Δ9 families e.g. DRG family 74 (Hutton *et al.* 1996; Perez-Tur *et al.* 1995k), and subsequently in other *PSEN1* families (Crook *et al.* 1998; Kwok *et al.* 1997; Smith *et al.* 2001; Verkkoniemi *et al.* 2000). This "cotton wool plaque" phenotype is associated with exceptionally high brain Aβ concentrations (Houlden *et al.* 2000; Steiner *et al.* 2001). In common with previous reports not all members of family 102 with FAD had evidence of spastic paraparesis (Crook *et al.* 1998; Kwok *et al.* 1997; Smith *et al.* 2001; Verkkoniemi *et al.* 2000) , suggesting that there must be genetic modifying factors affecting "classic" and "variant" expression within the same family. This is explored in a recently reported family with the E280Q mutation (i.e. same codon as family 102) which is similarly associated with spastic paraparesis and cotton wool plaques in some of the family members (Rogaeva *et al.* 2003). They

excluded an additional AD gene mutation and coding sequence variations in the three spastic paraparesis genes as being responsible for this genetic modifying factor.

In family 105/160 disease duration varied from 3 years to 15 years (mean 7.3 years), in family 102 the range was 10 years to 12 years (mean 11 years), and in family 177 the range was 6 years to 12 years (mean 8.3 years). This is consistent with previously published DRG *PSEN1* families (Fox *et al.* 1997; Kennedy *et al.* 1995b) and with predicted survival in sporadic AD cases (Bracco *et al.* 1994). Survival appears to be increasing in the later generations. Earlier diagnosis and better care undoubtedly contribute to this, but it is unclear in what proportion.

4.3.2 Neuropsychology

There is little neuropsychological data available for family 102. Patient 102.III.9 was assessed on the CAMCOG and obtained a score of 71/107 (Roth *et al.* 1986). Patients 105/160.IV.15 and 177.III.2 only underwent a limited assessment and presented with a global intellectual and memory impairment. Patient 177.III.3 was assessed twice. At the first assessment a mild global weakness in her memory functions was demonstrated by means of the verbal and visual versions of the RMT (Warrington 1984). At that time it was interpreted as being insufficient evidence for significant cognitive impairment. Today it would be interpreted as being consistent with (amnestic) Mild Cognitive Impairment (MCI) (Petersen *et al.* 2001). Unfortunately she was then lost to follow up for several years and when reassessed at the age of 41 years when she obtained a borderline defective Verbal IQ and a defective Performance IQ on the WAIS-R (Wechsler 1981). Her memory functions had gravely deteriorated; performance on the RMT for Words was at chance and on the RMT for Faces below the 1st percentile. Cases 105/160.V.16, 105/160.V.17 and 105/160.VI.08 all showed intellectual and memory deficits at the first assessment which became more pronounced with disease progression. Intellectual and memory failures have often been reported as a common feature of both familial and sporadic AD (Fox *et al.* 1997; Fox *et al.* 1998; Karlinsky *et al.* 1991; Lehtovirta *et al.* 1996; McKhann *et al.* 1984; Nee *et al.* 1983; Sadovnick *et al.* 1988). In the two less affected cases (105/160.V.16 and 105/160.V.17) verbal memory impairment preceded visual memory impairment. This finding is in accord with the notion that verbal memory is

more vulnerable than non verbal memory in FAD (Fox *et al.* 1997; Fox *et al.* 1998; Kennedy *et al.* 1995b; Newman *et al.* 1994).

Patients 177.III.3, 105/160.VI.08 and 105/160.V.16 presented with cognitive deficits extending beyond intellectual functioning and memory. In all three cases perceptual and spatial skills were more affected than naming skills. In patient 105/160.VI.16 perceptual and spatial skills deteriorated across assessments whilst naming skills remained static at a superior level. This pattern has previously been reported in a single FAD case, from a chromosome 14 linked pedigree (Newman *et al.* 1994) and our findings of relatively well preserved naming skills support the notion that this could be a common feature in FAD (Duara *et al.* 1993; Fox *et al.* 1997; Kennedy *et al.* 1995b; Newman *et al.* 1994; Warrington *et al.* 2001). Visuo-spatial and naming impairments have been reported in the context of mild SAD (Hodges & Patterson 1995; Jacobs *et al.* 1995; Kaskie & Storandt 1995; Morris *et al.* 1991).

In cases 105/160.V.16, 105/160.VI.08 and 177.III.3 calculation skills were more impaired than reading skills, a finding in accord with previous reports (Fox *et al.* 1997; Karlinsky *et al.* 1991; Kennedy *et al.* 1993; Kennedy *et al.* 1995b; Nee *et al.* 1983). In patient 105/160.V.16 spelling skills remained entirely preserved, whereas a spelling impairment was documented by Newman *et al.*(1994). It may be that these subtle differences between FAD pedigrees reflect disease severity or genotypic differences.

4.3.3 Neuroimaging

Patient 177.III.3 had a brain MRI at the time of her second assessment at the age of 41 years when her MMSE was 12/30. The MRI showed biparietal atrophy. In family 105/160 patient IV.15 had brain atrophy demonstrated indirectly by means of a lumbar air encephalogram. Cases 105/160.V.10 and 105/160.V.16 had brain CT scans, which demonstrated brains smaller than expected for age. These cases were already relatively far advanced in the disease process. Case 105/160.V.17 was imaged early in the disease process when her MMSE was still 27. Her brain MRI scan was reported as normal as were subsequent brain MRI scans 13 and 22 months later. Comparison of these scans however revealed evidence of early cerebral atrophy.

Cranial MRI performed on patients with *PSEN1* FAD typically reveals evidence of medial temporal lobe atrophy without significant white matter abnormalities. However, in family 102 two patients had brain MRI scans performed

that yielded rather different results to those seen in the other two families. Significant white matter abnormalities were seen on cranial MRI in patient 102.III.9 (see Figure 4.13), who had spastic paraparesis. Family member 102.III.3 also had white matter abnormalities on cranial MRI, in the absence of spastic paraparesis. Patient 102.III.2 also had spastic paraparesis, but unfortunately only had brain CT which was reported as normal. In the absence of significant risk factors for cerebrovascular disease, we propose that the white matter abnormalities seen on cranial MRI in these FAD patients most likely reflect an ischaemic leucoencephalopathy due to amyloid angiopathy affecting meningocortical vessels. This theory is supported by the prominent amyloid angiopathy on the brain biopsy specimen from patient 102.III.2. A previous study has shown no significant differences in white matter hyperintensity scores on cranial MRI between patients with EOAD and an aged-matched control group, when vascular risk factors were excluded (Scheltens *et al.* 1992a). There is a report of linear signal abnormalities within white matter in the parieto-occipital lobes of a patient with FAD due to *PSEN1* A285V (Aoki *et al.* 1997). Spastic paraparesis was not described and the lesions were attributed to cortical amyloid angiopathy.

4.3.4 Neuropathology

Neuropathological findings in patients 105/160.V.10, 105/160.V1.08 and 177.III.3 showed features of severe AD, including extensive formation of neuritic plaques, neurofibrillary tangles and neuropil threads. The deposition of β-amyloid was particularly prominent in the cortical ribbon. Patient 177.III.3 did not have amyloid angiopathy, but the two patients from family 105/160 had particularly severe amyloid angiopathy. In these two patients the amyloid was noted to spread from the vascular walls into the surrounding cerebral parenchyma to correspond to the classical picture of dyshoric angiopathy.

Although the general histological features of sporadic and familial cases of AD are the same, recent investigations have revealed differences in the severity of β-amyloid (Aβ) deposition, depending on the causative gene mutation. In cases due to APP717 mutations, the β-amyloid plaques are predominantly composed of $A\beta_{1\text{-}42(3)}$ with relatively little $A\beta_{1\text{-}40}$ present. The total amount of $A\beta_{1\text{-}42(3)}$ is considerably greater than in sporadic AD (Mann *et al.* 1996b). Similarly, in brains with FAD due to PSEN1 mutations, $A\beta_{1\text{-}42(3)}$ was the main component of plaques. Moreover, the total amount of

$A\beta_{1-42(3)}$ and $A\beta_{1-40}$ was more than twice the amount deposited in cases of sporadic AD of similar duration, although the ratio between the extent of the deposition of the two amyloid species was the same compared with sporadic Alzheimer's disease (Mann *et al.* 1996a). In a recent neuropathological study of 30 patients carrying 10 different presenilin 1 and 2 mutations, including patient 105/160.V.10, enhanced deposition of total β-amyloid and $A\beta_{1-42(3)}$, although not of $A\beta_{1-40}$, in the superior temporal gyrus was noted when compared to sporadic cases (Gomez-Isla *et al.* 1999). Moreover some of the *PSEN1* mutations (M139V, I143F, G209V, R269H and E280A) were also associated with faster rates of neurofibrillary tangle formation and an accelerated neuronal loss when compared with sporadic cases.

Cases 105/160.V.10 and 177.III.3 had significant Lewy body pathology in addition to the hallmark lesions of AD. In patient 105/160.V.10 several Lewy bodies were present in the pigmented nuclei of the brain stem and in restricted areas of the cerebral cortex whereas in patient 177.III.3 there was sufficient Lewy body pathology (see Figure 4.10) to consider a secondary diagnosis of dementia with Lewy bodies (transitional or limbic type) (McKeith *et al.* 1996). Although the co-existence of Lewy body and Alzheimer lesions is recognized in sporadic AD it is more common in FAD. In a series of 74 FAD cases 22% were complicated by Lewy bodies when using α-synuclein immunostaining to define them (Lippa *et al.* 1998). The Lewy body pathology in our cases, even 177.III.3, is negligible when compared with the Alzheimer changes, and there was no clinical Lewy body symptomatology to support the diagnosis. The Newcastle criteria could not be maintained in the light of α-synuclein immunostaining and have been revised (McKeith, Perry, & Perry 1999). For these reasons we feel a secondary diagnosis of dementia with Lewy bodies cannot be justified in either case.

The neuropathology of patient 102.III.2 was quite different to that of the other two families. Although only a brain biopsy specimen was obtained this revealed numerous, large, non-cored, "cotton-wool" plaques (see Figure 4.14). In this variant of FAD, although Aβ is deposited, almost no congophilic amyloid cores form in the brain parenchyma and therefore, as discussed in section 1.4.2, it has been postulated that the major determinant of dementia must lie upstream of these deposits in the pathogenic cascade (Crook *et al.* 1998; Kwok *et al.* 1997).

4.3.5 Molecular genetics

PSEN1 comprises 13 exons, of which exons 3-12 code for a 467 amino acid length protein (Clark *et al.* 1995e; Cruts *et al.* 1995). This serpentine protein is believed to comprise six to eight transmembrane (TM) domains, where mutations tend to be concentrated. The L153V mutation in family 177 is part of and extends a previously described mutation cluster on TM2 (see Table 4.6). The mutations in the TM2 cluster occur every third or fourth amino acid. Consequently it has been suggested that they line up on the same side of an α-helix, disrupting the structure and function of *PSEN1,* (Hardy 1997) a hypothesis that has since been extended for TM 1, 3, 4 and 6 (Hardy & Crook 2001).

The E280G mutation is located in the other major *PSEN1* mutation cluster which is in exon 8, near the PSEN1 cleavage site (Cruts, Hendriks, & Van Broeckhoven 1996). Together the TM2 and exon 8 mutation clusters account for over 50% of *PSEN1* mutations (Cruts & Van Broeckhoven 1998). The majority of *PSEN1* mutations have been missense mutations leading to the hypothesis that mutant proteins result in disease by toxic gain-of-function (Scheuner *et al.* 1996).

By contrast the *PSEN1* intron 4 mutation was thought to result in FAD by haploinsufficiency of full-length PSEN1 (Tysoe *et al.* 1998). However, it has since been shown that the intron 4 mutation produces three different transcripts, two deletion transcripts (PSEN1 Δ4 and $\Delta4_{cryptic}$) and one insertion transcript (ins_{TAC}), by aberrant splicing (De Jonghe *et al.* 1999b). De Jonghe and co-workers demonstrated that the latter transcript was due to the insertion of a TAC triplet between exons 4 and 5 of the PSEN1 (ins_{TAC}) and resulted in the insertion of a Threonine after amino acid 113 (T113-114ins) (De Jonghe *et al.* 1999b). In contrast to the insertion transcript, the truncated proteins were not detectable *in vivo* in brain homogenates or lymphoblast lysates of mutation carriers. Similarly De Jonghe and coworkers demonstrated that *in vitro* $A\beta_{42}$ secretion was increased in human embryonic kidney cells (HEK-293) overexpressing the insertion transcript, but not in those overexpressing the deletion transcripts. Increased $A\beta_{42}$:$A\beta_{40}$ ratios in brain homogenates were also demonstrated. Thus in common with previous missense mutations and in frame splice mutants, the pathogenic effect of the intron 4 mutation appears to be mediated by increased $A\beta_{42(3)}$ formation which is emerging as a general mechanism in presenilin and APP 717 mutation FAD (Duff *et al.* 1996; Scheuner *et al.* 1996).

Table 4.6 Presenilin 1 transmembrane domain-2 mutation cluster.

Mutation	Family Code	Mean Age at Onset (yrs)	Ethnicity	Reference
N135D	-	35	Mexican-American	(Crook *et al.* 1997)
M139I	AD1674	-	American	(Boteva *et al.* 1996)
M139K	ALZ 034	37	French	(Dumanchin *et al.* 1998)
M139T	CAE 010	49	French	(Campion *et al.* 1995b)
M139V	F148	44	English	(Clark *et al.* 1995e)
	F206	38	English	(Clark *et al.* 1995e)
	AD1421	-	American	(Boteva *et al.* 1996)
	-	40	German	(Sandbrink *et al.* 1996)
	#3	32	German	(Finckh *et al.* 2000)
	-	43	German	(Hull *et al.* 1998)
I143F	F156	55	English	(Rossor *et al.* 1996)
I143T	AD/A	35	Belgian	(Cruts *et al.* 1995)
M146I	-	44	Danish	(Jorgensen *et al.* 1996)
	-	-	American	(Cervenakova *et al.* 1996)
	-	43	Danish	(Essen-Möller 1946; Gustafson *et al.* 1998)
M146L	FAD4, Tor1.1	40, 43	Italian	(Sherrington *et al.* 1995m)
	Okla1	43	American	(Clark *et al.* 1995e)
	AR1	39	Argentinian	(Morelli *et al.* 1998)
	1,2,3	45,36,35	Italian	(Sorbi *et al.* 1995b)
	ALZ 204	43	French	(Campion *et al.* 1999)
M146V	Fin1	36	Finnish	(Clark *et al.* 1995e)
	Man92/20	40	English	(Clark *et al.* 1995e)
	NY5201	37	American	(Clark *et al.* 1995e)
T147I	ALZ 047	42	French	(Campion *et al.* 1999)
L153V	-	36	French	(Raux *et al.* 2000)
Y154C	F369	41	English	(Janssen *et al.* 2003)

5. A study of plasma amyloid-β peptide in familial Alzheimer's disease

5.1 Background

Familial AD was thought to be generally associated with an increased synthesis of more amyloidogenic $A\beta_{42}$. However, a number of important variations of APP processing have now been described in conditioned media: increased total Aβ synthesis ($A\beta_{40} > A\beta_{42}$) for Swedish *APP*, increased levels of both $A\beta_{40}$ and $A\beta_{42}$ for Flemish *APP*, an increased ratio of $A\beta_{42}$ to $A\beta_{40}$ for French *APP*, and reduced $A\beta_{42}$ levels for the three pathogenic mutations at codon 693 (Scheuner *et al.* 1996 ; Ancolio *et al.* 1999 ; Nilsberth *et al.* 2001). The ratio of the two Aβ isoforms seems as important as the absolute amount(s). In the Arctic *APP* mutation at codon 693 the generated amount of $A\beta_{42}$ is reduced, but it results in faster and greater protofibril formation than wild type Aβ, suggesting that protofibrils may accelerate the buildup of insoluble Aβ (Nilsberth *et al.* 2001). As prospects for AD disease modifying drugs improve, the value of biomarkers with surrogate and pre-symptomatic diagnostic potential becomes increasingly important. The advent of sensitive Aβ assays has stimulated interest in Aβ as an AD biomarker, particularly since it is found in both plasma and CSF. The presence of Aβ in plasma reflects synthesis in platelets as well as clearance from CSF. The latter may be reduced in AD, reflecting the lower CSF Aβ levels in AD patients. This chapter reports a small pilot study which investigates the suitability of plasma Aβ as a biomarker for FAD.

5.2 Subjects

All subjects were recruited from the longitudinal FAD study and from FAD patients attending the Specialist Cognitive Disorders Clinic at the National Hospital. All FAD patients and "at risk" family members were considered for inclusion in this study. Specific informed consent was obtained from each study entrant as previously outlined. Some family members had undergone predictive or diagnostic testing the results of which they had elected to make available allowing the patients to be categorized as follows: symptomatic FAD, pre-symptomatic FAD (positive predictive

genetic test), "at risk" (not genotyped) and non carriers (negative predictive genetic test).

5.3 Methods

Blood was collected into sodium ethylenediaminetetraacetic acid (EDTA) using polypropylene tubes and centrifuged immediately at 4000 rpm for three minutes. As soon as the centrifuge automatic locking mechanism allowed, supernatants were collected taking care to avoid disturbing the buffy coat. The plasma so obtained was then divided into aliquots (polypropylene tubes) of approximately 1 ml and immediately frozen in dry ice. The frozen samples were then transferred to a -70°C freezer until the time of analysis. These samples were then couriered to Dr Eric Southam (Glaxo Smith Kline, UK) on dry ice. The β-amyloid assays were performed by Dr Southam using commercially available enzyme linked immunosorbent assays (ELISA) without knowledge of diagnostic category. Plasma $A\beta_{(1-42)}$ was assayed using Innotest β-Amyloid $_{(1-42)}$ High Sensitivity Test (Innogenetics, Ghent, Belgium) a sandwich-type ELISA designed for the measurement of $A\beta_{(1-42)}$ in plasma and cell supernatants (Vanderstichele *et al.* 2000). Plasma $A\beta_{(1-40)}$ was determined using the BioSource Signal Select Human $A\beta_{(1-40)}$ ELISA kit (BioSource, Nivelles, Belgium).

5.4 Results

Twenty three patients were recruited: 11 "at risk" family members (7 *APP* and 4 *PSEN1*), 3 pre-symptomatic FAD patients (1 *APP* and 2 *PSEN1*), 6 FAD patients (2 *APP* and 4 *PSEN1*) and 3 non carriers. There was no difference in the age of the patients between the groups. Plasma $A\beta_{(40)}$ levels were almost identical in all groups (see Figure 5.1). Plasma $A\beta_{(1-42)}$ was higher in symptomatic FAD individuals (52.8 ± 3.2 $pg.ml^{-1}$) than family members who were non-carriers (29.6 ± 7.4 $pg.ml^{-1}$; $p<0.02$). Plasma $A\beta_{(1-42)}$ in pre-symptomatic FAD cases (55.2 ± 8.0 $pg.ml^{-1}$) tended to be higher than that in non-carriers ($p<0.1$) but was indistinguishable from symptomatic FAD (see Figure 5.2). Average plasma $A\beta_{(1-42)}$ concentrations in "at risk" family members (37.3 ± 2.2 $pg.ml^{-1}$) were midway between those of FAD mutation carriers (both symptomatic ($p<0.01$) and pre-symptomatic) and non-carriers. Unfortunately the small sample number dictated that *APP* and *PSEN1* mutations be grouped together (no obvious differences were apparent). The numbers of patients were too small to

test whether $A\beta_{(1-42)}$ levels changed with disease duration in patients or age in pre-symptomatic subjects (no obvious differences were apparent). Unfortunately *APOE* status was not known for the majority of subjects in this study.

5.5 Discussion

There was no difference in plasma $A\beta_{(40)}$ between the four groups of patients and this is in keeping with earlier observations. A degree of uncertainty surrounds many previous determinations of plasma $A\beta_{(42)}$ because most available assays lacked the required sensitivity. With the introduction of more sensitive assays greater consistency in plasma $A\beta_{(42)}$ determinations is expected to emerge. The number of individuals included in this study is small and the findings must be interpreted with caution. However, the observation that plasma $A\beta_{(42)}$ is elevated in those with FAD mutations, including those without symptoms, is supported by earlier work (Scheuner *et al.* 1996).

One of the more intriguing findings to emerge from this study was that the average plasma $A\beta_{42}$ concentration in at risk subjects was midway between the values obtained for carriers and non-carriers, perhaps suggesting that this group consisted of a combination of the two. It is, therefore, tempting to speculate that the individuals within this group who have the higher levels of $A\beta_{42}$ in their plasma are carriers of familial Alzheimer's disease mutations. As the genetic status of this group becomes clear, either by genotyping or by the emergence or otherwise of disease symptoms, it will be interesting to determine whether this turns out to be the case.

A previous study of two well characterised extended Belgian families AD/A and AD/B, respectively segregating *PSEN1* I143T and G384A, used the same sensitive $A\beta_{(1-42)}$ ELISA (De Jonghe *et al.* 1999a). Interestingly they showed that $A\beta_{(1-42)}$ levels detected in plasma were proportional to the individual *PSEN1* mutation's effect on $A\beta_{(1-42)}$ secretion in stable transfected human embryonic kidney cells (HEK-293). In family AD/A the I143T mutation resulted in only a modest increase in $A\beta_{(1-42)}$ secretion in HEK-293 cells and there was no difference in plasma $A\beta_{(1-42)}$ levels between mutation carriers, patients or escapees. However, in family AD/B there was a more than fivefold increase in $A\beta_{(1-42)}$ secretion in HEK-293 cells and a significant difference between mutation carriers, patients and escapees. Unfortunately the effect of individual family mutations on APP processing is not

known for the the patients included in this study, nor are their numbers sufficient to perform a similar analysis.

Levels of CSF $A\beta_{(42)}$ are reduced in sporadic AD (for reviews see Andreasen and Blennow 2002, and Verbeek, de Jong and Kremer, 2003), possibly due to sequestration in neuritic plaques, but it remains open to question whether $A\beta_{(42)}$ levels are elevated in their plasma (Fukumoto *et al.* 2003).

These preliminary results do suggest that plasma $A\beta_{(42)}$ concentrations may be a biomarker capable of identifying those individuals in whom FAD is present, even in the absence of symptoms, though that it may be dependent on the effect of individual FAD mutations on APP processing. It may have a role as a biomarker to facilitate the testing of novel anti-Alzheimer medicines, particularly in those whose symptoms are very mild or absent, providing the effect of their individual mutation on APP processing is known.

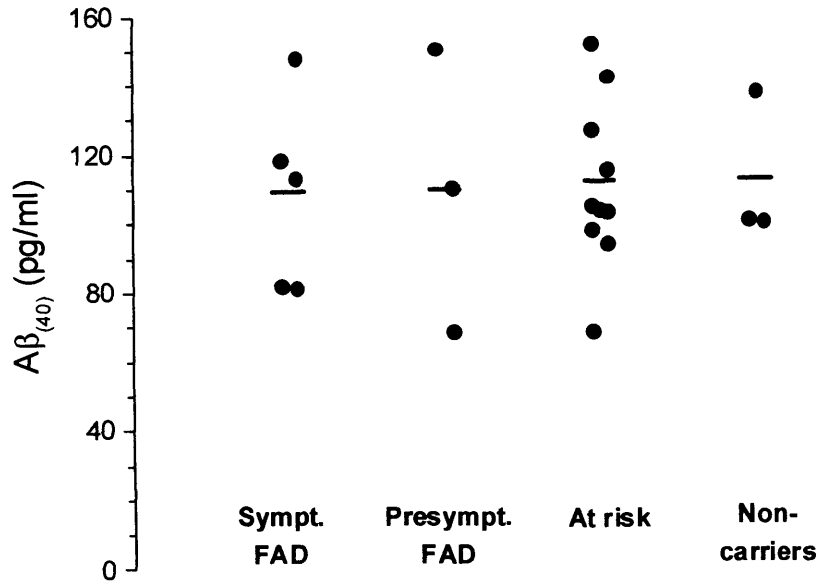


Figure 5.1: Plasma $A\beta_{(1-40)}$ in FAD cohort

(Mean values represented by a horizontal bar)

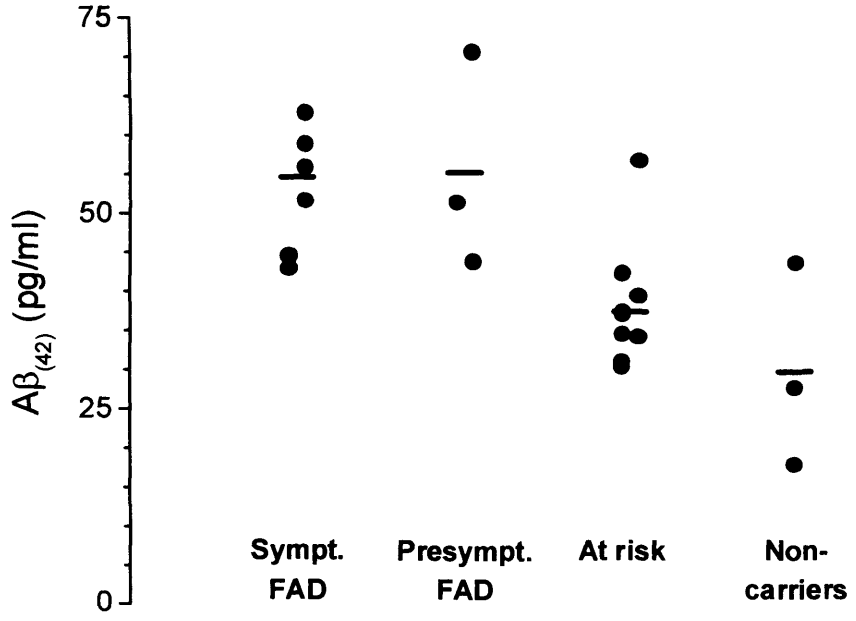


Figure 5.2: Plasma $A\beta_{(1-42)}$ in FAD cohort

(Mean values represented by a horizontal bar)

6. A volumetric MRI study of the progression of Alzheimer's disease

6.1 Background

The rate of cerebral atrophy in AD is significantly increased compared with age-matched control subjects. Furthermore there is considerable variation in the rate of brain atrophy between AD patients. One possible explanation for this degree of heterogeneity is that the different atrophy rates may reflect differences in the rates of clinical deterioration among AD patients, and in this context it is of note that rates of cerebral atrophy have previously been found to correlate with rates of change in MMSE scores in AD patients (Fox *et al.* 1999). Another possibility is that the rate of brain atrophy in AD might vary over time in line with the nature of the progression of the disease process. Accordingly changes in the rate of pathological involvement of the cerebral cortex would manifest as changes in the atrophy rate.

Previous longitudinal studies of AD have measured rates of brain atrophy across a single time interval (Chan *et al.* 2001a; DeCarli *et al.* 1992; Fox, Freeborough, & Rossor 1996; Jobst *et al.* 1994; Shear *et al.* 1995). Less is known about rates of cerebral atrophy occurring over sequential time intervals spanning several years. Demonstration of the changes in the rates of brain atrophy over time is important for understanding the natural history of AD and for assessment of disease modifying treatments.

In order to address the issue of longitudinal progression of brain atrophy in AD, the study in this chapter evaluated cerebral atrophy rates and ventricular enlargement in AD patients who have had multiple scans over a number of years.

6.2 Subjects and methods

Between January, 1992, and December, 1998, twelve early onset AD patients were recruited (see table 6.1). All patients provided written informed consent. The patients consisted of nine FAD patients from the longitudinal FAD study and three sporadic AD patients recruited through the Specialist Cognitive Disorders Clinic at the National Hospital for Neurology and Neurosurgery. All patients had probable AD (NINCDS-ADRDA criteria) by the end of the study (McKhann *et al.* 1984). In the

FAD group five patients were known to have *PSEN1* mutations, the remaining four having *APP* mutations. The sporadic AD patients had no family history of AD and previously performed *APP*, *PSEN1* and *PSEN2* mutation screening had been negative. The *APOE* genotype was known for all patients. In four FAD patients MRI scans were also obtained during the presymptomatic phase of the illness. All patients were assessed as previously described. The MMSE was used as a simple measure of dementia severity. Each patient underwent repeated MMSE testing, performed at the time of MRI scanning. All subjects were scanned on one of three 1.5T GE Signa MRI scanners (General Electric, Milwaukee, Wisconsin, USA) and T1-weighted volumetric images were obtained using a spoiled gradient echo technique as previously described to provide 124 contiguous 1.5mm thick slices in the coronal plane. All repeat scans were performed on the same scanner.

Only scans without substantial movement or intensity inhomogeneity problems were analysed. Image data were transferred to a Sun workstation (Sun Microsystems, Mountain View, California, USA). The scans were registered using a nine degrees-of-freedom algorithm, incorporating three linear scaling factors, to compensate for interscan variations in voxel dimensions (Fox & Freeborough 1997). Rates of whole brain atrophy were calculated following registration of each subject's pair of scans. Rates of atrophy were measured using three different techniques:

- First, the total brain volume (TBV) was delineated from CSF using a semi-automated process based on a signal intensity thresholding algorithm, with non-brain structures excluded by manual editing. Volume loss was represented by a difference in TBV across two scans. TBV measurements were corrected for total intracranial volume (TIV), to control for potential changes in voxel size due to scanner drifts over time, and to allow comparisons between individuals with different head sizes (Whitwell *et al.* 2001). All TIVs in this project were measured by Jennifer Whitwell.
- Second, cerebral atrophy was determined using the previously described and validated BBSI technique, which calculates from each registered (positionally matched) scan pair the shift in the position of the brain-CSF boundary, integrated over the entire brain surface to provide a measure of volume loss (Fox & Freeborough 1997).

- Third, atrophy was assessed by measuring enlargement of ventricular CSF volume corrected for TIV. Ventricles were measured by me as previously described (see Appendix 4) and corrected for TIV to control for potential changes in voxel size and to correct for head size (Whitwell *et al.* 2001).

6.3 Statistical analysis and modelling

At the time of the initial scan the severity of AD in individual patients ranged from the presymptomatic stage of disease through to moderate dementia. To assess changes in atrophy rates with varying disease severity, atrophy rates were documented relative to the time when every patient obtained an MMSE score of 23/30, a score representing mild dementia and attained by all patients during clinical/research follow-up. Statistical analysis was performed by Chris Frost and Hillary Watts.

To allow for repeated within individual measurements, a hierarchical mixed quadratic regression model relating brain volume (as a proportion of TIV) to time (relative to when MMSE = 23), was used to determine the variation in atrophy rates over time. All modelling was done in *MLwiN* (*MLwiN*, Institute of Education, University of London). Brain volumes were log-transformed to model percentage changes. Linear and intercept terms were allowed to vary between individuals (random effects) while the quadratic term was fixed, with the logarithm of the rate of change of percentage yearly brain loss given by twice this term. This variation of linear and intercept terms allowed for true interindividual variability and for any inaccuracy in specifying the time when MMSE equalled 23.

A related model was used for BBSI-derived changes in volume. The outcome variable was the logarithm of the ratio of brain volumes at the end and start of follow-up, with the ratio at the end of this period calculated from the BBSI and brain volume at the start. This BBSI-derived percentage change in brain volumes was related to the difference in visit times (random effects) and the difference in visit times squared (fixed effects). The model allowed for errors introduced during measurement of changes and at the beginning and end of the measurement periods. Equations for both models are given in Figure 6.1.

The same hierarchical mixed quadratic regression model used to relate brain volume (as a proportion of TIV) to time (relative to when MMSE = 23) was also used to determine changes in ventricular CSF volumes over time.

6.4 Results

At the time of first MRI the mean age of the patients was 47 years (SD 7.8; range 35 – 59) and mean MMSE was 26 (range 16 - 30). The male: female ratio was 5:7. The average period from first to last scan was 43.2 months (range 15.8 – 66.3). The mean number of usable scans per patient was 5.2 (SD 2.4; range 3-11). In the nine patients with FAD four were known to have *APP* mutations, and five had *PSEN1* mutations. Apolipoprotein E 3/3 and 3/4 genotypes were found in seven and five patients respectively.

6.4.1 Cerebral atrophy

Atrophy rates were similar whether estimated from directly measured brain volumes (Figure 6.2; Table 6.2) or BBSI. The change in rates (ie, rise per year in annual percentage brain volume loss) was estimated to be 0.32% (95% CI 0.15-0.50) from measured brain volumes and 0.31% (95% CI: 0.19 - 0.43) from BBSI.

6.4.2 Ventricular enlargement

Ventricular CSF volumes increased with time as shown in Table 6.2, with an estimated acceleration of 0.009% (95% CI: 0.0000 – 0.0175; P= 0.05) per annum.

6.4.3 Effect of genotype

In view of the borderline significance for ventricular enlargement only cerebral atrophy measures were assessed for the effects of *APOE* and FAD genotypes (Table 6.3). Comparing *APOE* ε4/ε3 with ε3/ε3 revealed no differences, with similar results for TBV and BBSI. There was no difference in atrophy rates between the FAD and SAD patients, again with similar results for TBV and BBSI.

6.5 Discussion

Individual patients exhibited a gradual acceleration in cerebral atrophy rates and ventricular enlargement from the presymptomatic stages through to moderately severe disease. The relation between the two measures of whole brain change accords with previous work showing that BBSI values equate to about 80% of brain volume loss

(Fox & Freeborough 1997). Previous studies have shown that atrophy is higher in patients with greater disease severity, which could be consistent with acceleration (Murphy *et al.* 1993; O'Brien *et al.* 2001).

Although there was borderline evidence that the rate of ventricular enlargement increases i.e. a non-linear trend, this was not as convincing as for whole brain derived measurements. This would suggest that cerebral tissue atrophies faster than ventricles enlarge. This may be due to the relative absolute volumes, but it seems more likely that this is because ventricular enlargement reflects only a small proportion of cerebral atrophy. It does, however provide further evidence that whole brain derived measures are more sensitive than ventricular measurements.

This is the first study to use multiple timepoints and between-individual and within-individual changes in atrophy rates to assess changes in atrophy rates. Pronounced between-individual variability in atrophy rates was also demonstrated, with a two-fold variation in atrophy rates between patients, as derived from fitted values at the time MMSE equalled 23. This finding is in keeping with the clinical variability in the rate of functional decline, which has been shown to correlate with rates of cerebral atrophy (Fox *et al.* 1999).

There were no statistically significant differences in rates of cerebral atrophy between patients with FAD and SAD, nor according to *APOE* genotype. This is perhaps unsurprising since the number of patients is modest, so moderate sized differences in the rates could be missed. The estimated rate of atrophy is somewhat higher in FAD compared to SAD, and interestingly somewhat lower in those with *APOE* 3/4 genotype compared to *APOE* 3/3 genotype. The confidence intervals reflect the limits of uncertainty. So although the demonstrated between-individual differences in cerebral atrophy may have a genetic basis, the small size of the cohort did not permit assessment of the effect of specific genotypes on rates of atrophy.

Enrolling patients with early onset AD facilitated serial assessment and scanning over several years. The fact that the nine FAD patients had a definite genetic diagnosis of AD adds validity to the study findings. However, caution must be exercised in extrapolation of these results to the entire population with AD: first, the natural history of late onset sporadic AD might differ from that noted in this study; second, these results were based on data obtained during the early stages of AD and therefore do not necessarily apply to the severe disease stage. These results could

indicate true acceleration of atrophy in already affected brain regions, disease spread to a greater proportion of the cerebral cortex, or both.

The acceleration in atrophy was apparent only over several years; over one to two years, the progression of atrophy was approximately linear. The relative constancy of the atrophy rate over shorter time periods is important for the use of cerebral atrophy rates as biomarkers of disease progression. The near-linearity of atrophy progression can be used to model AD treatment effects over periods of less than two years, with attenuation of disease progression in treated patients represented by a reduction in brain atrophy rate. Increasing rates of atrophy, as demonstrated in this study, means that trials attempting to demonstrate a drug effect on AD progression would possess greater statistical power, and would require smaller sample sizes, by enrolling moderately affected rather than mildly affected patients.

Finally, the demonstration that neuronal loss is already evident at a presymptomatic stage of AD, and that there is a subsequent accelerating cascade of tissue atrophy, reinforces the need for early diagnosis and therapeutic intervention in AD.

Table 6.1 Study patient demographics

ID	Sex	Diagnosis	ApoE	Gene	Age at onset (yrs)	No. scans	T1 (days)	T2 (days)	MMSE1	MMSE2
DB	M	FAD	3/3	PS1	39	6	-527	1195	23	25
HB	F	FAD	3/3	PS1	35	3	551	480	26	16
CG	F	FAD	3/3	PS1	39	5	1821	1002	16	0
AH	F	FAD	3/4	APP	50	8	1538	1616	23	7
CL	F	FAD	3/3	PS1	39	11	-962	2016	30	25
VL	F	SAD	3/3	N/A	55	6	2357	1766	24	15
JM	M	FAD	3/4	PS1	48	7	2437	1678	25	8
DN	M	FAD	3/3	APP	56	5	1138	847	27	15
AS	F	FAD	3/4	APP	48	5	-447	1970	30	17
KS	F	SAD	3/4	N/A	53	3	1171	602	27	26
WU	M	SAD	3/3	N/A	59	4	977	1260	22	16
AW	M	FAD	3/4	APP	47	4	-97	1343	19	11

Patient details: M = male; F = female. FAD = familial AD; SAD = sporadic AD. T1 = time of initial scan relative to estimated date of symptom onset; T2 = time of final scan relative to first scan. MMSE1 = MMSE score at time of initial scan; MMSE2 = MMSE score at time of final scan.

Table 6.2 Estimated annual rates of progression

Time from MMSE of 23/30 (years)	Brain atrophy rate (% per year) derived from TBV* measurements	Brain atrophy rate (% per year) derived from BBSI measurements	Percentage Rate of increase in Ventricles/ TIV
-2	2.17 (1.58 - 2.75)	1.60 (1.23 - 1.96)	0.173 (0.132 to 0.214)
-1	2.49 (1.97 - 3.00)	1.90 (1.59 - 2.21)	0.181 (0.143 to 0.220)
0	2.80 (2.32 – 3.29)	2.21 (1.91 - 2.50)	0.190 (0.153 to 0.227)
1	3.12 (2.60 - 3.64)	2.51 (2.84 - 2.18)	0.199 (0.161 to 0.237)
2	3.44 (2.84 – 4.03)	2.81 (2.41 - 3.21)	0.208 #

Table 6.3 Effect of genotype on rates of cerebral atrophy

Comparison groups	Measurement used to determine atrophy	Difference in rate of atrophy as percentage of brain volume lost per year (95% CI)
FAD v. SAD	TBV*	0.10 (-1.24 to 1.41)
FAD v. SAD	BBSI	0.33 (-0.49 to 1.14)
APOE 3/4 v *APOE* 3/3	TBV*	-0.32 (-1.37 to 0.72)
APOE 3/4 v *APOE* 3/3	BBSI	-0.24 (-0.85 to 0.37)

FAD = familial AD, SAD = sporadic AD, TBV* = total brain volume corrected for total intracranial volume, BBSI = brain boundary shift integral, # = CI could not be estimated

Figure 6.1 Equations used for modelling atrophy

Results were derived from fitting the following two equations:
Log of brain volume over TIV for subject i at visit j:

$$\log\left(Volume_{ij}/TIV_{ij}\right)=(\alpha+a_i)+(\beta+b_i)t_{ij}+j\,\delta t_{ij}^2+e_{ij}$$

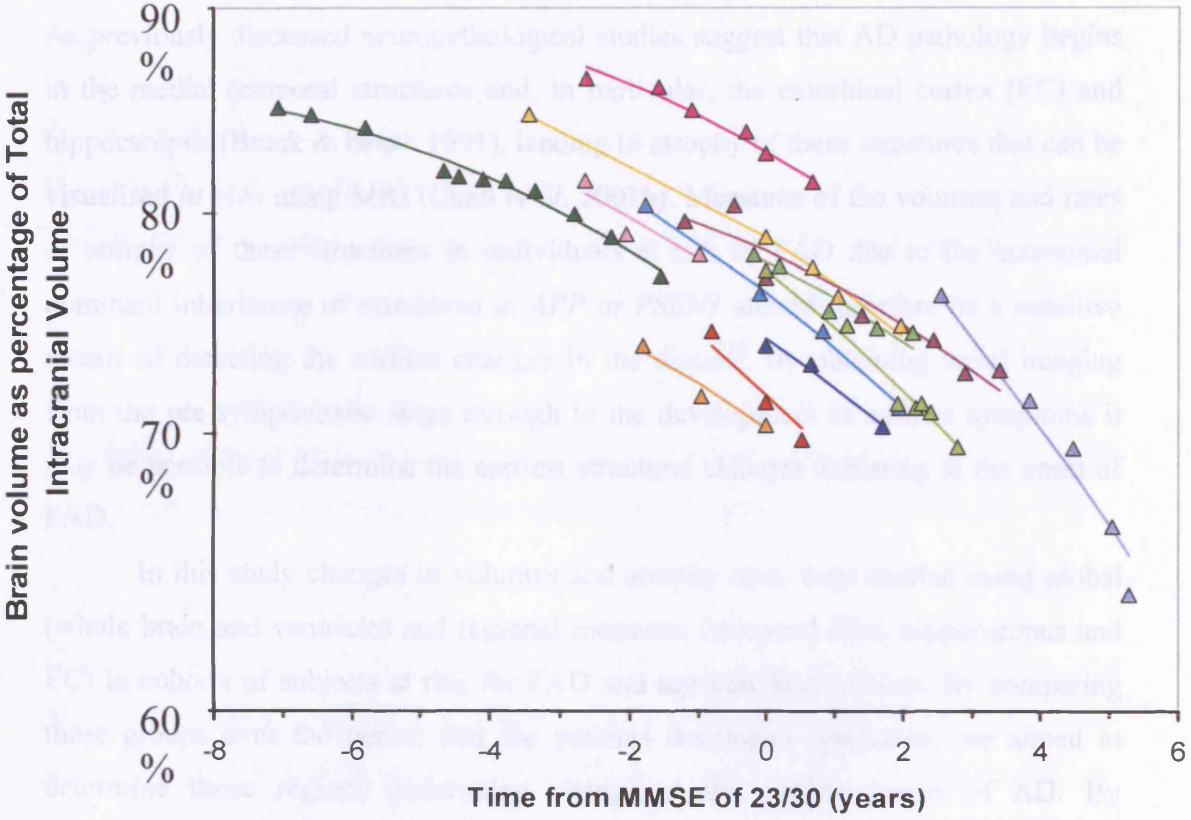
where t_{ij}=time of ith scan for participant j and $e_{ij} \sim N(0,\sigma^2)$ and $\begin{pmatrix}a_i\\b_i\end{pmatrix} \sim N\left[\begin{pmatrix}0\\0\end{pmatrix},\begin{pmatrix}\sigma_a^2 & \sigma_{ab}\\ \sigma_{ab} & \sigma_b^2\end{pmatrix}\right]$

BBSI measured difference between brain volumes in time points j and k for participant i:

$$\log\left(\frac{Volume_{ij}-BBSI_{ijk}}{Volume_{ij}}\right)=(\beta+b_i)(t_{ik}-t_{ij})+\delta(t_{ik}^2-t_{ij}^2)+u_{ik}-u_{ij}+d_{ijk}$$

where $b_{ijk} \sim N(0,\sigma_b)$, $u_{ij} \sim N(0,\sigma_u^2)$, and $d_{ijk} \sim N(0,\sigma_d^2)$

Figure 6.2 Progression of brain atrophy derived from total brain volume corrected for intracranial volume



Progression in rates of brain atrophy over time, as determined by serial measurements of total brain volume, represented as a proportion of total intracranial volume. Patients are individually colour-coded. Time is plotted relative to the time (t=0) when a score of 23/30 was obtained on MMSE.

7. A volumetric MRI study of the site of earliest change in familial Alzheimer's disease

7.1 Background

As previously discussed neuropathological studies suggest that AD pathology begins in the medial temporal structures and, in particular, the entorhinal cortex (EC) and hippocampus (Braak & Braak 1991), leading to atrophy of these structures that can be visualised *in vivo* using MRI (Chan *et al.* 2001b). Measures of the volumes and rates of atrophy of these structures in individuals at risk of FAD due to the autosomal dominant inheritance of mutations in *APP* or *PSEN1* should therefore be a sensitive means of detecting the earliest changes in the disease. By obtaining serial imaging from the pre-symptomatic stage through to the development of earliest symptoms it may be possible to determine the earliest structural changes occurring at the onset of FAD.

In this study changes in volumes and atrophy rates were studied using global (whole brain and ventricle) and regional measures (temporal lobe, hippocampus and EC) in cohorts of subjects at risk for FAD and age-matched controls. By comparing these groups over the period that the patients developed symptoms, we aimed to determine those regions undergoing change at the earliest stages of AD. By extrapolation, we estimated how long prior to symptom onset medial temporal lobe atrophy had occurred.

7.2 Subjects and Methods

7.2.1 Subjects

All subjects were seen at the National Hospital for Neurology and Neurosurgery, Queen Square, London. The study received local ethics committee approval, and all subjects gave informed, written consent. As part of the longitudinal study individuals at risk of developing FAD underwent annual MR imaging, standardised MMSE and detailed clinical and neuropsychological assessments as previously described. During this time period five subjects (three male, two female) exhibited early symptoms of the disease and eventually went on to fulfil NINCDS-ADRDA criteria for the diagnosis of probable AD. All have since been shown to carry FAD mutations (two in

APP; three in *PSEN1*). Two patients had four MRI and clinical assessments, one patient had three assessments and two patients had two assessments; all exhibited progressive cognitive impairment over the period of investigation, and went on to fulfil NINCDS-ADRDA criteria for the diagnosis of probable AD. Twenty age-matched controls had two serial volumetric MR scans and MMSE assessments.

7.2.2 MRI acquisition and registration

T1-weighted volumetric MR brain scans were acquired on a 1.5 Tesla Signa unit (General Electric, Milwaukee, USA), as previously described. This sequence yielded 124 contiguous 1.5mm coronal slices, which were transferred to a Sun workstation (Sun Microsystems Inc., Mountain View, CA., USA) for analysis using the MIDAS image analysis program as previously described (Freeborough, Fox, & Kitney 1997). Axial T2-weighted scans were also acquired. For each subjects, follow-up scans were performed on the same scanner, and accurately registered onto baseline images, using a nine degrees of freedom rigid body registration protocol.

7.2.2 Volumetric analysis

With the exception of whole brain and ventricular segmentation, baseline and registered repeat scans were analysed as a pair for each subject. The first segmentation was performed using the MIDAS package. The resulting region was then pasted onto the remaining scan and edited using intensity thresholding and manual tracing. To prevent potential laterality bias, the scans were flipped across the mid sagittal line to produce two scans, each a mirror-image of the other; segmentation was always performed on the same side of the presented image. Each substructure was outlined by a single investigator who was blind to diagnosis, left-right orientation, and to whether the scan was the baseline or repeat image.

Total intracranial volume (TIV) was measured according to a previously described protocol and was used to normalize for differences in head size (Jenkins *et al.* 2000). Rates of whole brain atrophy were calculated directly from the TIV corrected brain volumes, and using the Brain Boundary Shift Integral (BBSI) as described earlier. Mean intra-rater reproducibility (calculated as the mean value of the absolute difference between a pair of measurements divided by their mean for each segmented region) for the investigators, using the protocols described below, was:

whole brain segmentation and ventricles <1%; for temporal lobes and hippocampi <3%; and for EC 5%.

7.2.3 Brain segmentation

Whole brain segmentation (see Appendix 3) was performed using a semi-automated iterative morphologic technique with the threshold for the boundary between brain and CSF set at 60% of mean brain image intensity. The inferior cut-off for the brain volume was taken through the brainstem at the level of the most inferior point of the cerebellum. Each brain region was then checked and manually edited where necessary.

7.2.4 Ventricular segmentation

Ventricular volumes were measured (see Appendix 4) on the baseline and repeat images for each subject using 60% image intensity thresholds calculated from each brain region. Ventricular volumes included the lateral ventricles and temporal horn of the lateral ventricles but not the third or fourth ventricle.

7.2.5 Temporal lobe segmentation

A threshold of 60% of mean brain intensity was employed to improve consistency of cerebrospinal fluid-brain delineation. The posterior boundary of the temporal lobe was taken to be the slice where the longest length of the fornix was visible (Chan *et al.* 2001b). One temporal lobe region was outlined on all slices including temporal lobe tissue. This region was then pasted on to the second image and edited to accommodate any structural changes.

7.2.6 Hippocampal segmentation

A consistent threshold of 70% of mean brain intensity was applied for hippocampal segmentation. Measurements were taken from coronal slices from the posterior to anterior boundaries using a standard neuroanatomical atlas (Duvernoy & Bourgoin 1998). The posterior limit of the hippocampus was defined as the coronal slice where the longest length of the fornix is seen (Watson *et al.* 1992). This measurement method excludes the posterior part of the hippocampal tail in order to achieve satisfactory reproducibility of segmentation. The rostral border was taken to be at the

junction with the amygdala. The hippocampus was bounded superiorly, medially, and laterally by the ambient cistern, and inferiorly by the subjacent white matter.

7.2.7 Entorhinal cortex segmentation

Segmentation of the entorhinal cortex (EC) was undertaken using a modification of the protocol of Insausti *et al.* (Insausti *et al.* 1998). White matter was not included within this measurement. Rostrally the EC extended as far as the rostral extreme of the sulcus semiannularis and the caudal end of the EC was taken as the end of the gyrus infralimbicus. Superiorly the EC was bounded by the white matter separating it from the amygdala (rostrally) and the hippocampus (caudally). The inferomedial boundary of the EC was taken to be the ambient cistern, and laterally the EC was measured as far as the medial lip of the collateral sulcus. The portion of the EC that extended along the medial bank of the collateral sulcus was not included in the measurement because of the variation between individuals in the position of the lateral border of the EC.

7.2.8 Statistical methods

Whole brain volume and volumes of brain regions were standardised to the geometric mean TIV in controls. This was done by fitting linear regression models to the control patients (both visits) relating each volume (whole brain, ventricular, left and right temporal lobe, left and right hippocampal, left and right entorhinal) to TIV, with all variables on logarithmic scales.

Analyses of all TIV adjusted volumes other than those of the ventricles were carried out on a logarithmic scale. Baseline TIV adjusted volumes were compared between cases and controls using unpaired t-tests allowing for unequal variances in cases and controls. Comparisons were made for whole brain, ventricle, left and right temporal lobe, left and right hippocampus and left and right EC volumes, together with the geometric mean of all four hippocampal and EC volumes. Ratios of left to right EC volumes and left to right hippocampal volumes were calculated for each subject and compared between cases and controls, as was the ratio of the geometric means of the (TIV adjusted) EC volumes and hippocampal volumes.

Percentage changes in whole brain volume were estimated both as the ratio of the BBSI to the baseline brain volume, and directly from the TIV-corrected brain volumes. Absolute changes in ventricular volume, percentage changes for the other

structures, the geometric mean for the combined hippocampal and EC volumes, the ratios of the left to the right EC and hippocampal volumes and the ratio of the geometric mean of the EC volumes to that of the hippocampal volumes were calculated from the directly measured volumes. Atrophy rates were calculated by relating changes (on a log-scale for proportions) to follow-up time. Atrophy rates were compared between cases and controls using unpaired t-tests allowing for unequal variances in cases and controls. The ratios of left to right EC atrophy rate, left to right hippocampal atrophy rate and EC to hippocampal atrophy rates were also compared using unpaired t-tests allowing for unequal variances in cases and controls.

Mean baseline levels and atrophy rates in cases and controls were used to extrapolate to 'points of divergence' under the assumption of linear (on a logarithmic scale) rates of decline prior to the start of follow-up. Under this assumption the point of divergence is equal to the ratio of the difference in mean levels at baseline to the difference in atrophy rates. A slightly modified version of Fieller's theorem (Buonaccorsci 1998; Fieller 1940) that allows for the relatively small number of subjects was used to calculate a confidence interval for these ratios. The modification involved the replacement of the 97.5 percentile of the Normal distribution with that from a t-distribution with the same number of degrees of freedom as used in the t-test comparing baseline levels for each structure in the confidence interval formulae. All statistical analyses were carried out using STATA version 6.0 (Stata Corp., College Station, TX, USA).

7.3 Results

During the course of the study, all five of the at-risk subjects developed probable Alzheimer's disease according to conventional NINCDS-ADRDA criteria. Table 7.1 shows characteristics of these five cases, and those of the 20 control subjects.

7.3.1 Brain and brain sub-structure volumes at baseline

Whole brain and substructure volumes (ventricles, temporal lobes, hippocampi and EC) were calculated from the baseline scan, and are shown, corrected for differences in TIV in Table 7.2. There were no significant differences in ventricular, left temporal lobe and right temporal lobe volumes between patients and controls. Both left and right hippocampal volumes were lower in cases than controls, although only the 18%

difference in right hippocampal volumes achieved statistical significance (p=0.002). EC volumes were significantly lower in patients than controls (left, p=0.009; right, p=0.04). There was no evidence of EC asymmetry and the difference between disease-associated reduction in hippocampal and EC volumes was also not statistically significant. Averaging results from the four hippocampal and EC volumes resulted in an estimated overall difference of 16.6% (p=0.03) between patients and controls.

7.3.2 Atrophy Rates

Figure 7.1 shows the TIV adjusted whole brain, ventricular and left and right temporal lobe volumes for each subject at each follow-up visit. Subject specific volumes are expressed relative to mean baseline level in cases and controls. Figure 7.2 shows equivalent data for left and right hippocampal and left and right EC volumes. In the three subjects with more than two scans, there is little indication of non-linearity.

Table 7.3 shows atrophy rates for patients and controls and the differences between them for each brain region. In controls, there was a small but statistically significant reduction in whole brain volume. Using both BBSI and TIV corrected brain volumes whole brain annual atrophy rates were 1% greater in patients than in controls. This difference was statistically significant (p=0.004) for the BBSI derived atrophy rates. There was a significant increase in the measure of ventricular volume increase for both controls (0.44ml per year, $p<0.05$) and patients (2.35ml per year, $p<0.001$). The difference between the rates in cases and controls was also significant ($p<0.05$)

For all measured brain regions, atrophy rates were significantly increased in the patients compared with controls ($p<0.05$). For both left and right temporal lobe volumes, the difference in rates was around 2% per year, whilst for both the left and right hippocampus it was around 3% per year, with no evidence of asymmetry in rates. The largest differences were observed for the EC. The atrophy rate for the right EC (8.9% per year) was greater than that for the left (4.9% per year) but this difference did not achieve statistical significance (p=0.1). Combining results from the left and the right (TIV adjusted) volumes EC atrophy rates were statistically significantly greater than hippocampal rates in the AD cases (5.7% per year greater, p=0.02). However EC atrophy rates were also statistically significantly greater than hippocampal rates in controls (1.7% greater, $p<0.001$) and comparison of the excess

rate in cases with that in controls gave a borderline statistically significant result (p=0.056). Averaging results for the four hippocampal and EC atrophy rates, resulted in an overall difference of 5.05% (p=0.002) between cases and controls.

Figure 7.3 illustrates the construction of the point of divergence for the averaged medial temporal lobe measurements. At baseline, measurements in cases were on average already 16.6% below that in controls with a rate of decline that was 5.05% greater than that in controls. Making the assumption that atrophy rates were constant prior to the start of follow-up this suggests an average point of divergence 3.5 years prior to the start of the study, at which time all patients were asymptomatic. Confidence intervals around this and the points of divergence for whole brain and the individual structures are however wide (Table 7.4).

7.4 Discussion

These results show that measures of temporal lobe sub-structure volumes, and atrophy rates derived from longitudinal scanning can distinguish groups of patients with very early AD from controls.

At the commencement of the study, both left and right hippocampal and left and right EC volumes were already smaller in cases than in controls. Combining results from these four medial temporal lobe regions showed that volumes were 16.6% lower in cases than controls. By contrast whole brain volumes and ventricular size were similar between patients and controls. These findings imply that medial temporal lobe atrophy must be occurring prior to symptom onset, and as such are in concordance with previous DRG studies (Fox *et al.* 1996; Scahill *et al.* 2002). Pathological studies suggest that the EC should be expected to show more prominent volume changes than the hippocampi early in the disease process (Braak, Braak, & Bohl 1993). Imaging data support this, with relatively greater losses in the EC than in the hippocampus when mild AD (Bobinski *et al.* 1999) and "questionable AD" subjects (Killiany *et al.* 2000; Killiany *et al.* 2002) were compared with controls. The results reported here were directionally consistent with this hypothesis but the effect was not statistically significant. The lack of statistical significance probably reflects a lack of power resulting from the small numbers in this study, the relatively large inter-subject variability and errors inherent in boundary definition, reflected by the wide confidence intervals. The higher measurement error for EC (5%) volume measures

compared with that for the hippocampus (3%) is evidence for the increased difficulty in reliably defining the EC. Differences in measurement reliability led Xu *et al.* (Xu *et al.* 2000) to conclude that despite theoretical considerations, there was no practical benefit of EC over hippocampal measures in distinguishing patients with MCI, early AD and controls; a conclusion that may be altered if improved image acquisition and analysis make the EC easier to measure.

This study has demonstrated significant increases in whole brain atrophy as measured using both the BBSI and a measure of increased ventricular volume in normal controls. These findings are likely to reflect the inevitable mild global atrophy (secondary to neuronal loss) associated with normal ageing, even in this age-group (Pakkenberg & Gundersen 1997); it is likely that these changes would be larger still in a more elderly cohort. However, such changes are clearly different in scale to the significantly greater atrophy seen in AD as demonstrated in several previous longitudinal imaging studies (Fox & Freeborough 1997; Luxenberg *et al.* 1987). This is confirmed in this study of younger subjects with FAD, with excess whole brain atrophy occurring in the pre-symptomatic AD group at a rate of around 1% per year. The study was not designed to detect differences in atrophy rates between regions in controls. Nonetheless it is interesting to observe that the largest atrophy rates are seen in the EC, although given the small number of subjects and the wide confidence intervals this result needs to be viewed with some caution.

Significantly increased rates of temporal lobe, hippocampal and EC atrophy were demonstrated in patients compared to controls, indicating that regional atrophy, particularly affecting medial-temporal lobe structures occurs very early in the disease process. Such longitudinal comparisons, by using patients as their own controls, are less likely to be influenced by inter-individual differences than cross-sectional analyses, even if in the latter corrections are made for TIV. Although not significantly different, the fact that rates of atrophy for the EC are considerably higher than those from the hippocampus or temporal lobe, suggests that EC atrophy rates might be the most sensitive method of discriminating early AD from controls.

This study was not designed to assess all candidate regions within the brain. Whilst it is recognized that medial temporal lobe structures bear the brunt of the disease process in early AD, recent imaging studies have highlighted that neocortical areas, and in particular the posterior cingulate cortex may also be involved from the earliest stages of the disease (Baron *et al.* 2001; Callen *et al.* 2001; Scahill *et al.*

2002). Such regions have proved difficult to delineate and quantify reliably, and at present medial temporal lobe volumes and atrophy rates continue to be more widely assessed as potential diagnostic and disease progression measures in AD.

As discussed in Chapter 6 there is evidence to suggest that rates of atrophy accelerate as the disease progresses. This also seems to be true for earliest stages of AD (Jack *et al.* 2000), which is biologically plausible given that there must be a transition from the atrophy rates of normal ageing to the increased rates described here. If rates of atrophy increase very gradually, then this accelerating degenerative process may have begun very many years earlier. If however, one assumes that initial rates of atrophy had been constant in the period prior to the start of volume measurement a minimum estimate of the onset of pathological atrophy may be calculated. Using this model, with the assumption that rates of medial temporal lobe atrophy are constant at this early stage the calculated mean time from the start of medial temporal lobe atrophy to the start of the study was 3.5 years (see Figure 7.3). The 95% confidence interval around this estimate (0.7 – 7.5 years) is wide and this result must be interpreted cautiously. Of the patients reported two had baseline MMSE scores below 27, probably indicative of the presence of early cognitive impairment. However, all subjects were thought to be asymptomatic at the start of follow-up, and would not have come to medical attention without a family history FAD. The wide confidence intervals reported in this study are therefore likely to in part reflect subtle differences in disease stage, even prior to symptom manifestation

Extrapolating these results to all patients with AD requires considerable caution, since these calculations are based on a small number of patients with FAD. Not only are these patients considerably younger that patients with sporadic AD (SAD), but the progression of their disease may also be different. Indeed, in addition to differences between FAD and SAD, there are also phenotypic differences between FAD patients with different mutations. However, within the older SAD group there is considerable heterogeneity and the advantage of the FAD cohort is the certainty of diagnosis, the lack of co-morbidity and the feasibility of assessment from a presymptomatic stage.

Table 7.1: Characteristics of subjects

	Subject characteristics (mean ± SD)			
	Age (years)	***Baseline MMSE***	***Final MMSE***	***Follow-up (days)***
Cases (n=5, 3 male)	42.9 ± 4.5	28.4 ± 2.3	25.6 ± 4.3	864 ± 244
Controls (n=20, 10 male)	45.8 ± 6.8	29.4 ± 0.8	29.3 ± 0.8	515 ± 293

Table 7.2: Mean baseline volumes in 'at risk' subjects

Brain Region	*Geometric mean volumes in cases (N=5) as a % of that in controls (N=20) at baseline, after adjustment for differences in total intracranial volume (95% confidence intervals)*
Whole Brain	98.2 (95.1, 101.5)

Left Temporal Lobe	101.3 (93.9, 109.2)
Right Temporal Lobe	98.9 (92.4, 105.9)

Left Hippocampus	92.1 (81.4, 104.1)
Right Hippocampus	81.9 (70.9, 94.6)
Left Entorhinal Cortex	76.7 (55.0, 107.0)
Right Entorhinal Cortex	83.7 (71.1, 98.6)
Mean	**83.4 (71.8, 97.0)**

Brain Region	*Mean volumes (ml) in cases (N=5) and controls (N=20) at baseline, after adjustment for differences in total intracranial volume (95% confidence intervals)*		
	Cases (N=5)	*Controls (N=20)*	*Difference*
Ventricles	13.2 (9.9, 16.4)	14.3 (10.2, 18.4)	-1.1 (-5.9, 3.6)

Table 7.3: Annual changes in measured brain regions

Brain Region	*Percentage change in volume per year*		
	Cases (N=5) Rate (95% CI)	*Controls (N=20) Rate (95% CI)*	*Additional change in cases (95% CI)*
Whole brain:			
using baseline +BBSI	-1.23 (-1.72, -0.74)	-0.28 (-0.41, -0.14)	-0.96 (-1.44, -0.48)
using TIV correction	-1.08 (-2.64, 0.49)	-0.12 (-0.63, 0.39)	-0.96 (-2.48, 0.58)
Left Temporal Lobe	-2.08 (-3.37, -0.78)	-0.13 (-0.54, 0.27)	-1.95 (-3.21, -0.68)
Right Temporal Lobe	-1.95 (-3.10, -0.79)	0.05 (-0.27, 0.37)	-2.00 (-3.13, -0.86)
Left Hippocampus	-2.76 (-4.66, -0.82)	0.45 (-0.26, 1.17)	-3.19 (-5.05, -1.30)
Right Hippocampus	-3.31 (-5.98, -0.57)	-0.21 (-1.51, 1.12)	-3.11 (-5.76, -0.38)
Left Entorhinal Cortex	-5.97 (-7.63, -4.24)	-1.16 (-2.34, 0.03)	-4.86 (-6.64, -3.05)
Right Entorhinal Cortex	-10.73 (-16.21, -4.89)	-1.99 (-3.10, -0.86)	-8.92 (-14.44, -3.04)
Mean	**-5.74 (-7.83, -3.62)**	**-0.73 (-1.24, -0.22)**	**-5.05 (-7.11, -2.95)**

Brain Region	*Change in mls per year*		
	Cases (N=5) Rate (95% CI)	*Controls (N=20) Rate (95% CI)*	*Additional change in cases (95% CI)*
Ventricles	2.35 (0.71, 4.00)	0.44 (0.04, 0.84)	1.91 (0.31, 3.52)

Table 7.4: Estimated points of divergence (95% CI) in atrophy rates relative to baseline

Brain Region	*Estimated points of divergence (years) relative to start of follow-up (95% confidence interval)*
Whole Brain	-1.83 (-4.05, 2.55)
Ventricles	0.59 (-1.78, 5.87)
Left Temporal Lobe	0.64 (-3.70, 5.88)
Right Temporal Lobe	-0.54 (-4.05, 3.85)
Left Hippocampus	-2.55 (-7.75, 1.60)
Right Hippocampus	-6.32 (-409.12, -1.48)
Left Entorhinal Cortex	-5.32 (-17.42, 1.19)
Right Entorhinal Cortex	-1.90 (-5.63, -0.15)
Mean	**-3.50 (-7.54, -0.66)**

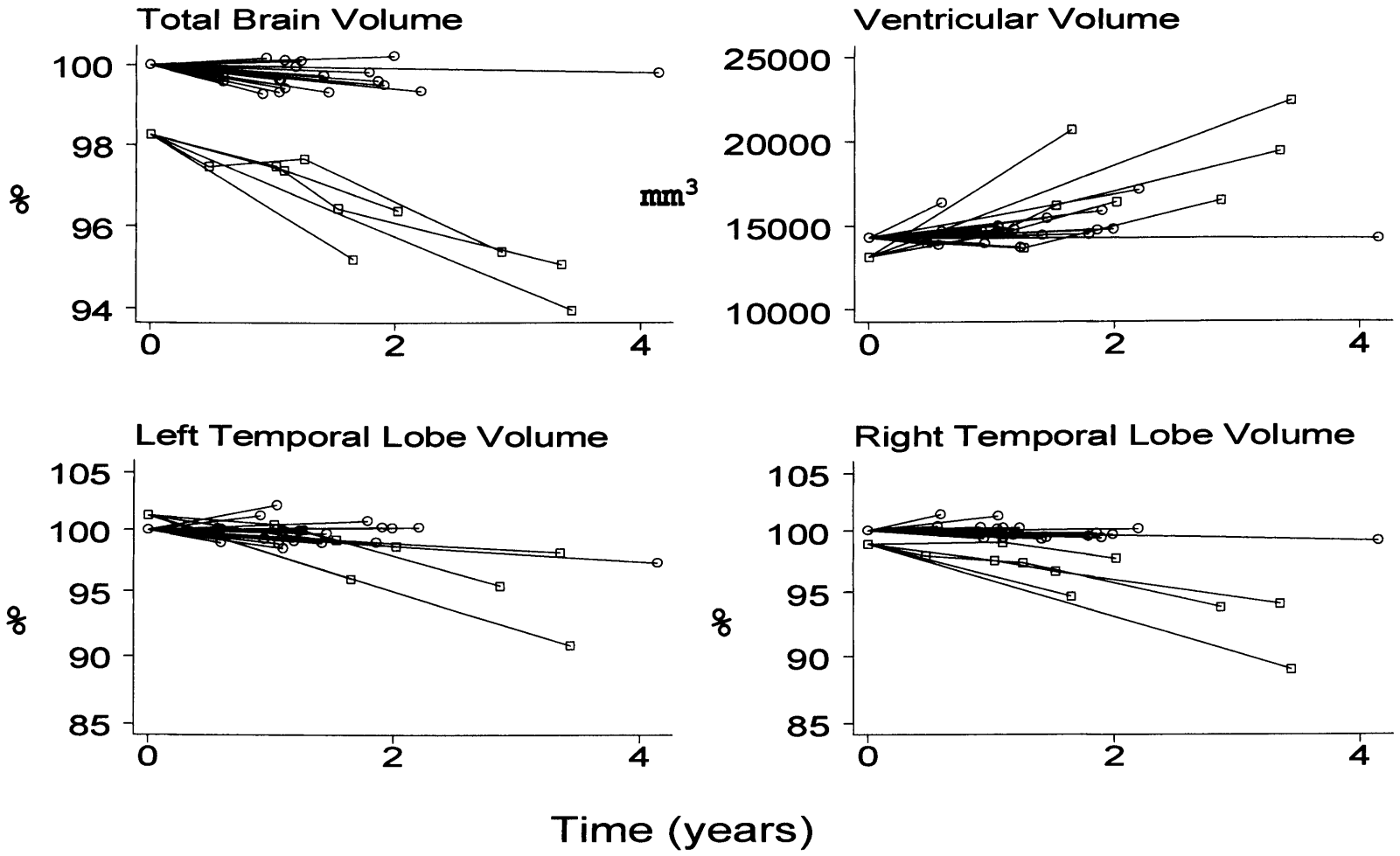


Figure 7.1: **Brain volumes (brain, ventricles and temporal lobes) over time presented relative to mean baseline levels.** All volumes corrected for total intracranial. Squares represent cases (n=5) and circles controls (n=20).

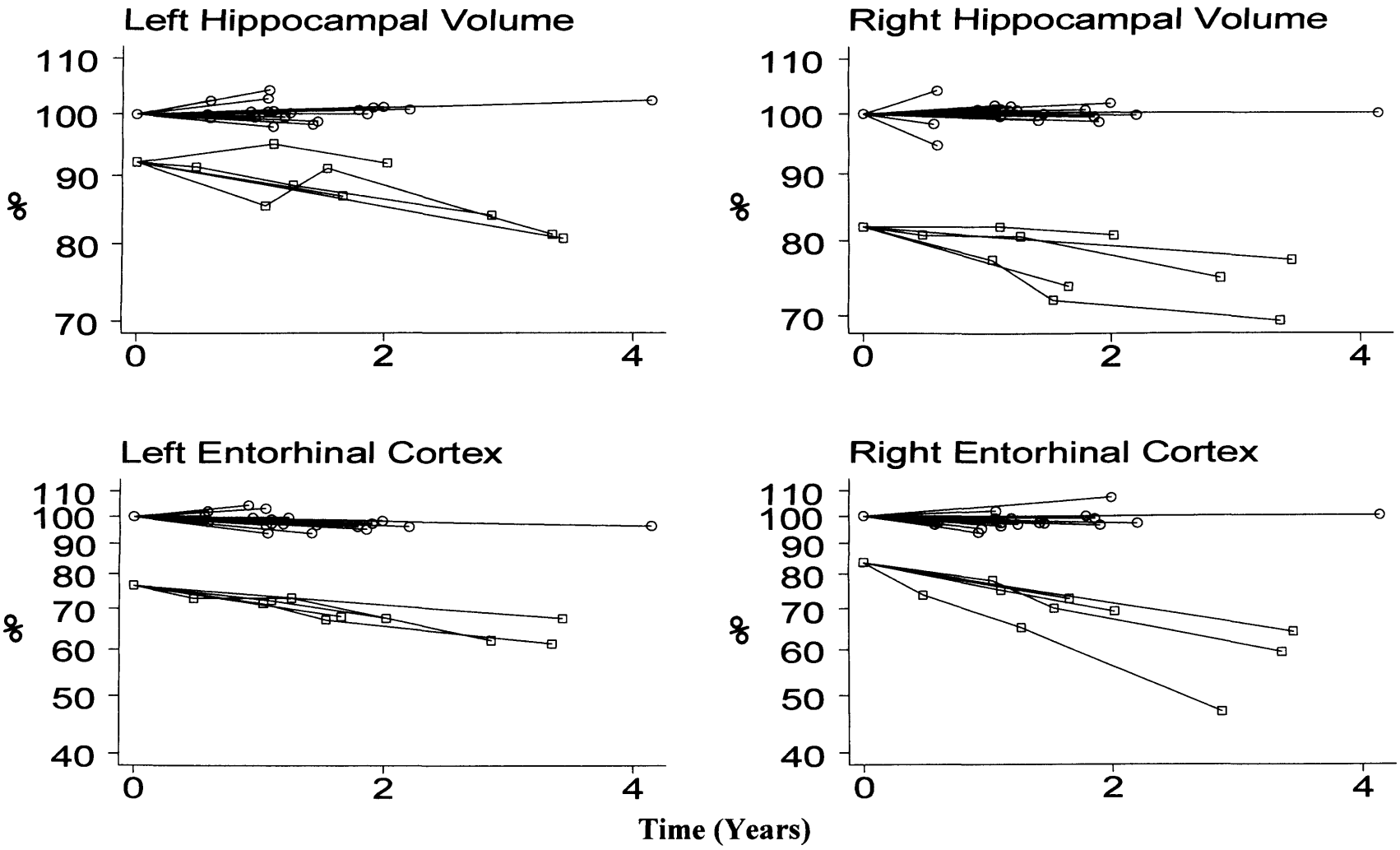


Figure 7.2: **Brain volumes (hippocampal and entorhinal cortex) over time presented relative to mean baseline levels.** All volumes corrected for total intracranial. Squares represent cases (n=5) and circles controls (n=20).

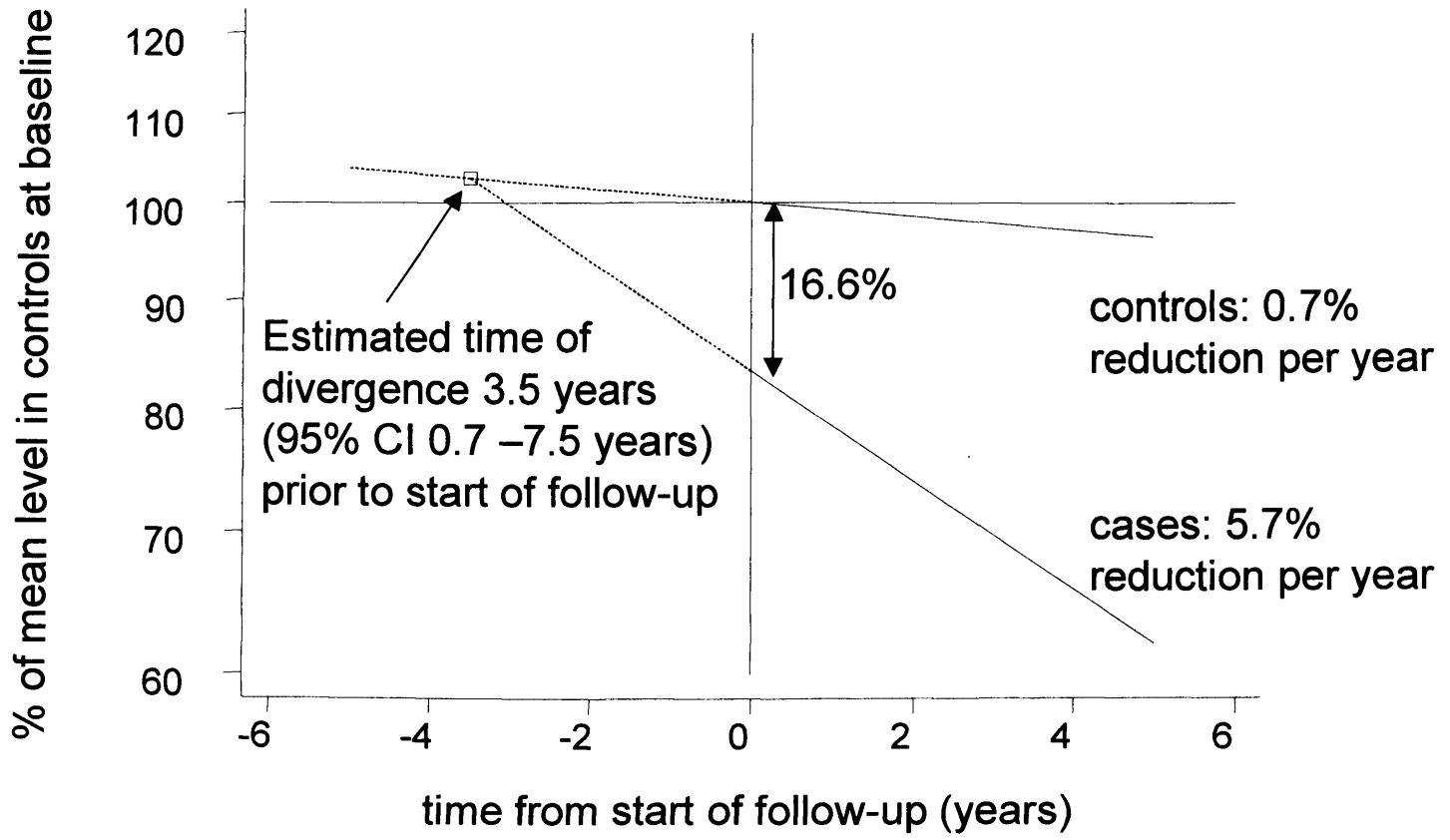


Figure 7.3: **Construction of the 'point of divergence' for medial temporal lobe volume.**
(Medial temporal lobe volume = geometric mean of all four hippocampal and entorhinal cortex volumes).

8 The onset and progression of Alzheimer's disease imaged with voxel compression mapping of serial MRI

8.1 Background

The study presented in the previous chapter assessed medial temporal lobe structures which from cross-sectional studies are thought to bear the brunt of the histopathological changes in Alzheimer's disease (AD) (Braak & Braak 1991). However, because of these *a priori* assumptions Chapter 7 could not address where the disease process of familial AD (FAD) begins nor how it progresses, which is important for developing therapeutic strategies aimed at prevention. To answer this question requires longitudinal follow-up of individuals from a presymptomatic stage using *in vivo* measures. To achieve this previous studies have included those at higher risk of "converting" to AD either by enrolling those with mild cognitive impairment (Petersen *et al.* 2001) or by recruiting elderly individuals who were APOEε4 hetero/homozygotes (Reiman *et al.* 1996; Small *et al.* 1995). The "at risk" offspring of patients with FAD are at 50% risk of the disease at a relatively predictable age at onset. These individuals provide a group that may be studied longitudinally with a reasonable chance of identifying the earliest manifestations of the disease. Comorbidity such as vascular disease is rare in these individuals because of their young age at disease onset. Furthermore because of their known genetic risk of AD the diagnosis is relatively secure in life without histological confirmation (Fox *et al.* 1998). Although there are differences between FAD and sporadic AD, most notably an earlier age at onset in FAD, the significant clinical, pathological and neuropsychological similarities support the use of FAD as a model of the disease that is amenable to presymptomatic study.

8.2 Introduction

Neuronal damage at the cellular level is accompanied by macroscopic structural readjustments: deformations, positional shifts as well as volume loss. This complex

and dynamic process is inadequately captured with a single imaging snapshot of the brain. Serial imaging adds a fourth dimension, change over time, to 3D MRI. Sensitive methods are necessary to visualise and quantify subtle brain changes that may have occurred over the interval between scans. Digital subtraction of the serial imaging provides that sensitivity as long as accurate positional matching - registration - is first achieved. Rigid-body registration methods are now capable of bringing serial imaging into sub-voxel alignment, allowing meaningful subtraction of an individual's later scan from their earlier scan. Registration that treats the brain as a rigid body can match the brain as a whole and can be used to quantify whole brain atrophy (Freeborough, Woods, & Fox 1996). However, because of structural readjustment, the sites of tissue loss cannot be accurately determined. A non-linear registration algorithm is needed in order to track local cerebral losses and deformations (Sowell *et al.* 1999). A compressible viscous fluid model provides a plausible model of the structural changes associated with cerebral degeneration and has previously been used to visualise developmental change (Thompson *et al.* 2000). Non-linear registration methods, incorporating this model, can also be used to match serial MR scans from individuals with degenerative dementia (Freeborough & Fox 1998). This matching provides voxel-by-voxel maps of volume change, Voxel Compression Maps, that can localise regions of atrophy.

8.3 Methods

Subjects

Twenty clinically diagnosed individuals with sporadic and familial AD, fulfilling NINCDS-ADRDA criteria (McKhann *et al.* 1984), were recruited from the Specialist Cognitive Disorders Clinic at The National Hospital for Neurology and Neurosurgery; median (range) age 53 (38-76) years and median initial mini-mental state examination (MMSE) (Folstein, Folstein, & McHughs 1975) 22/30 (8-27). Twenty healthy control subjects were recruited from patients' spouses; median age 51 (39-71) years, median initial MMSE 30/30 (28-30). Four individuals at risk of FAD from the DRG longitudinal study, who were initially asymptomatic but subsequently became affected during follow-up, were also studied. These subjects came from FAD families with known autosomal dominant mutations (*APP* V717G, *PSEN1* M139V and *PSEN1* intron 4). However, none of the at-risk individuals nor the researchers involved in the clinical or MRI assessments knew any individual's mutation status. All subjects were

assessed annually with full history, examination and comprehensive neuropsychometry as described earlier (Newman *et al.* 1994). Informed consent was obtained from all subjects and the study had the approval of the Local Research Ethics Committee.

At baseline the median age of the at-risk subjects was 43.4 (39-51) years and they had MMSE scores of 27, 29, 29 and 30/30. The at-risk subjects were asymptomatic in that neither they nor their close informants felt there were symptoms of memory loss or other cognitive decline. Performances on stringent tests of verbal and visual recognition memory (Warrington 1984) were normal apart from one individual who scored below the fifth percentile on both, who nonetheless fulfilled entry criteria as asymptomatic. Other cognitive functions were normal on the previously described extensive battery of neuropsychological tests. The four subjects were followed for between five and eight years and all became symptomatic and subsequently progressed to fulfill established criteria for AD. Two individuals developed symptoms of memory loss by the time of their second scan and the other two developed symptoms 1-3 years after their second scan.

Magnetic resonance imaging acquisition

Serial, approximately annual imaging, was acquired on all subjects together with additional scans with intervals varying from hours to months. Three-dimensional T1-weighted MRI were performed on a 1.5 Tesla Signa unit (General Electric, Milwaukee) using a fast SPGR (spoiled gradient-recalled acquisition in the steady state) sequence yielding 124 contiguous 1.5mm coronal slices of 256 x 256 voxels (acquisition parameters: TR/TE/NEX/FLIP - 35/5/1/35).

Image postprocessing

Scans were presented for analysis in a random order, and performed by operators blind to the subject's details. As a pre-processing step the brain was segmented from non-brain structures in each scan using a semi-automated algorithm followed by manual editing (Freeborough, Fox, & Kitney 1997). For each pair, the later scan was accurately aligned to the baseline scan using an automatic image registration technique which optimised the match for brain to sub-voxel accuracy (Freeborough, Woods, & Fox 1996). In the case of multiple scans all repeat scans were registered to the baseline image. A voxel scaling correction was applied during registration to

accommodate variation in the calibration of scanner voxel dimensions (Freeborough, Woods, & Fox 1996). Accurate measures of global brain atrophy were computed using the Brain Boundary Shift Integral (Fox & Freeborough 1997).

Fluid matching algorithm

Local structural changes were detected by matching the rigidly registered image pairs using a highly non-linear registration algorithm (Freeborough & Fox 1998). This registration uses a viscous fluid model to compute a voxel-level deformation field constrained to be continuous, topology-preserving and one-to-one. The computation is iterative and can be completed in a few hours on a contemporary desktop workstation. Voxel Compression Maps can be derived from the deformation field by computing the determinant of the Jacobian matrix at each point to provide a point-estimate of volumetric change. These maps are combined with the structural images as colour overlays to allow association of patterns of change with neuroanatomically significant regions (See Figure 8.2).

8.4 Results

The rigid body registration and subtraction revealed cerebral volume losses in all subjects over time. The median (range) global rates of loss in the subjects with mild to moderate AD were significantly greater than those in the healthy controls, 2.20 (0.82-4.19) % vs 0.24 (-0.35-0.64) % per year (Wilcoxon rank-sum, $p<0.001$). The median initial rate of atrophy for the four at-risk individuals was 1.0 (0.78-1.88) % per year, which was significantly greater than the control rate (Wilcoxon rank-sum, $p<0.001$). In addition the subtraction images confirmed that in AD the brain undergoes considerable deformation and positional shifts as atrophy progresses, for example the corpus callosum elevates and individual gyri are subject to rotational deformations. Fluid registration was able to resolve local structural brain changes and demonstrate far superior matching to rigid body registration (see Figure 8.1). Derived voxel compression maps were markedly different between the AD cases and normal controls (see Figure 8.2). In regions on the MR images where the image appears homogenous and featureless such as cerebrospinal fluid within the ventricles, the volume changes are appropriately shown to be diffuse. Consistent patterns of loss were seen in the AD cases with both widespread grey and white matter regions undergoing diffuse volume losses. These losses were marked in medial temporal lobe

and neocortical association areas and by contrast, the primary motor and sensory cortices, brain stem and cerebellum were spared (see Figure 8.3). The small global volume losses seen in the healthy controls were shown in the voxel compression maps as diffuse loss with minor enlargement of both sulcal and ventricular spaces. As a control, fluid registration and voxel compression mapping were also applied to pairs of scans of affected individuals acquired on the same day, which confirmed the stability of the technique in that little overall change was visible compared with intervals of months or years.

The four at-risk subjects had increased rates of cerebral atrophy prior to fulfilling the clinical criteria for Probable AD. Furthermore, fluid registration of images spanning the onset of symptoms revealed diffuse losses involving the medial temporal lobe but also showed increased rates of atrophy in the infero-lateral temporal lobe, the parietal lobe and the posterior cingulate (see Figure 8.4). In one at-risk individual a series of 12 scans spanning a three year symptom-free phase and four years of cognitive decline had been acquired. Analysis of the presymptomatic scans suggest accelerating atrophy rates starting five years prior to diagnosis (see Figure 8.5). Even at the very earliest stages, increased atrophy with involvement of the medial temporal lobe was present. As the individual became clinically affected, atrophy rates increased and changes became widespread, eventually becoming comparable to the patterns and rates seen in established sporadic AD.

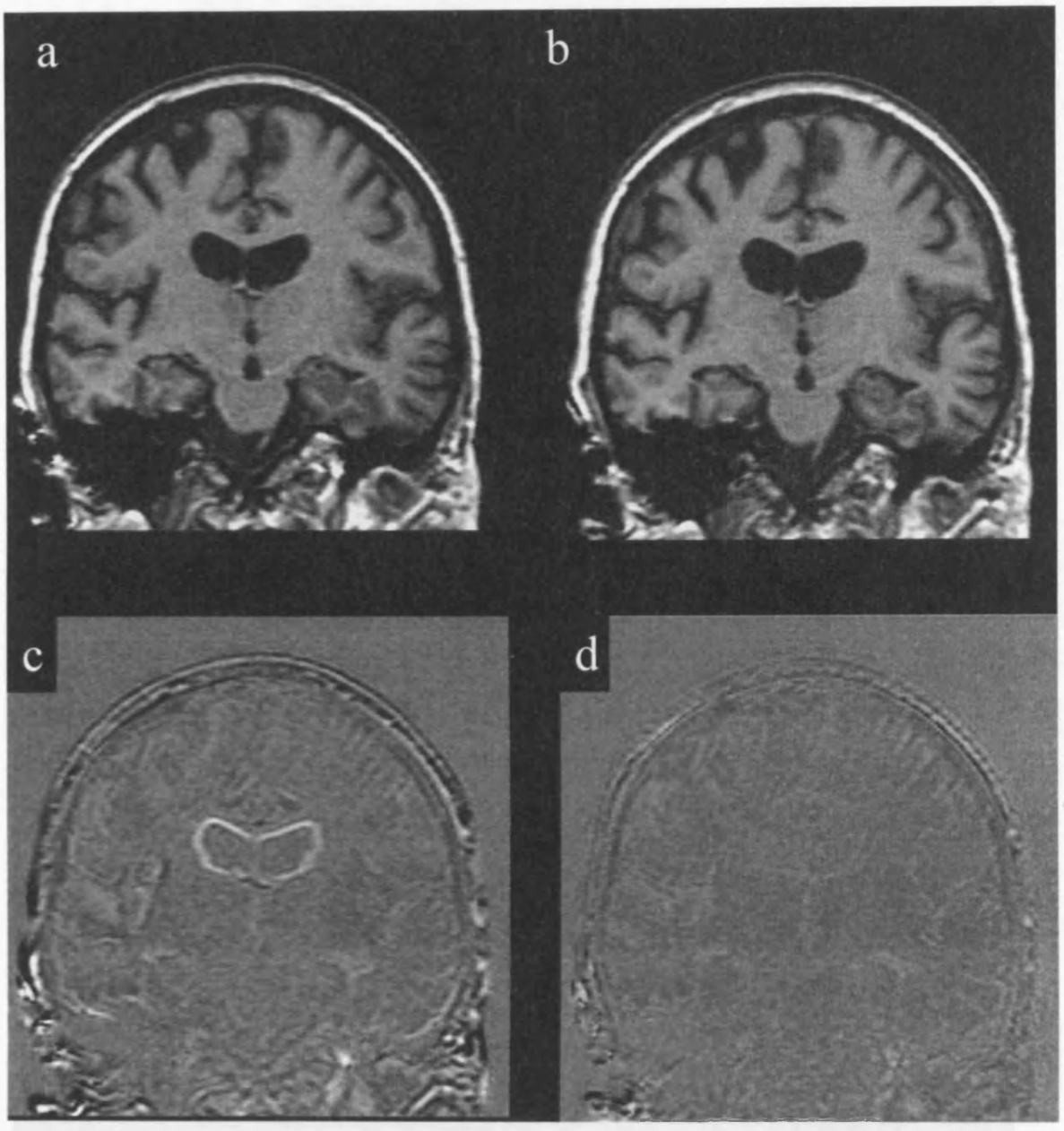
8.5 Discussion

The distribution of atrophy in both preclinical and established AD as revealed by voxel compression mapping accords well with the pattern of cognitive deficits and the distribution of observed histological changes at autopsy. Braak and Braak (1991) reported neurofibrillary tangles in the hippocampus and entorhinal cortex in elderly controls at post-mortem and more extensive medial temporal lobe changes in mildly memory impaired subjects. If these cross-sectional data are viewed as a continuum this implies the locus of initiation of Alzheimer's disease is in the medial temporal lobe. The voxel compression mapping provides longitudinal data compatible with this. Although cytoskeletal changes, i.e. neurofibrillary tangles and neuritic plaques, are rare in the primary cortices and cerebellum, diffuse plaques and amyloid deposition are not, suggesting that the neuritic component of the histopathological changes are essential for neuronal disintegration and atrophy. The reason for the

topographical vulnerability is not clear.

The presymptomatic individuals represent a unique cohort because serial imaging was acquired many years before they would have presented clinically. However some caution is needed in generalising results found in FAD to the more common sporadic AD. The finding of increased rates of atrophy in the subjects in this study is consistent with evidence of significant hippocampal atrophy in very mild sporadic and familial AD and in minimal cognitive impairment (de Leon *et al.* 1993b; Jack *et al.* 1997).(de Leon *et al.* 1993b) Subtle memory defects may predate the diagnosis of AD by several years (Linn *et al.* 1995) and abnormalities in cerebral glucose metabolism or brain activation during memory tasks have been reported to be detectable even earlier in cognitively normal individuals with a family history of Alzheimer's disease or with a increased genetic risk of AD (Bookheimer *et al.* 2000; Kennedy *et al.* 1995a; Reiman *et al.* 1996; Small *et al.* 1995). We cannot be certain when structural changes are first detectable but it appears that atrophy rates increase several years before symptoms. This study cannot address the issue of when histological changes (plaques, tangles and cell loss) start but it seems reasonable to suggest that they are likely to precede the macroscopic, atrophic changes demonstrated in this study.

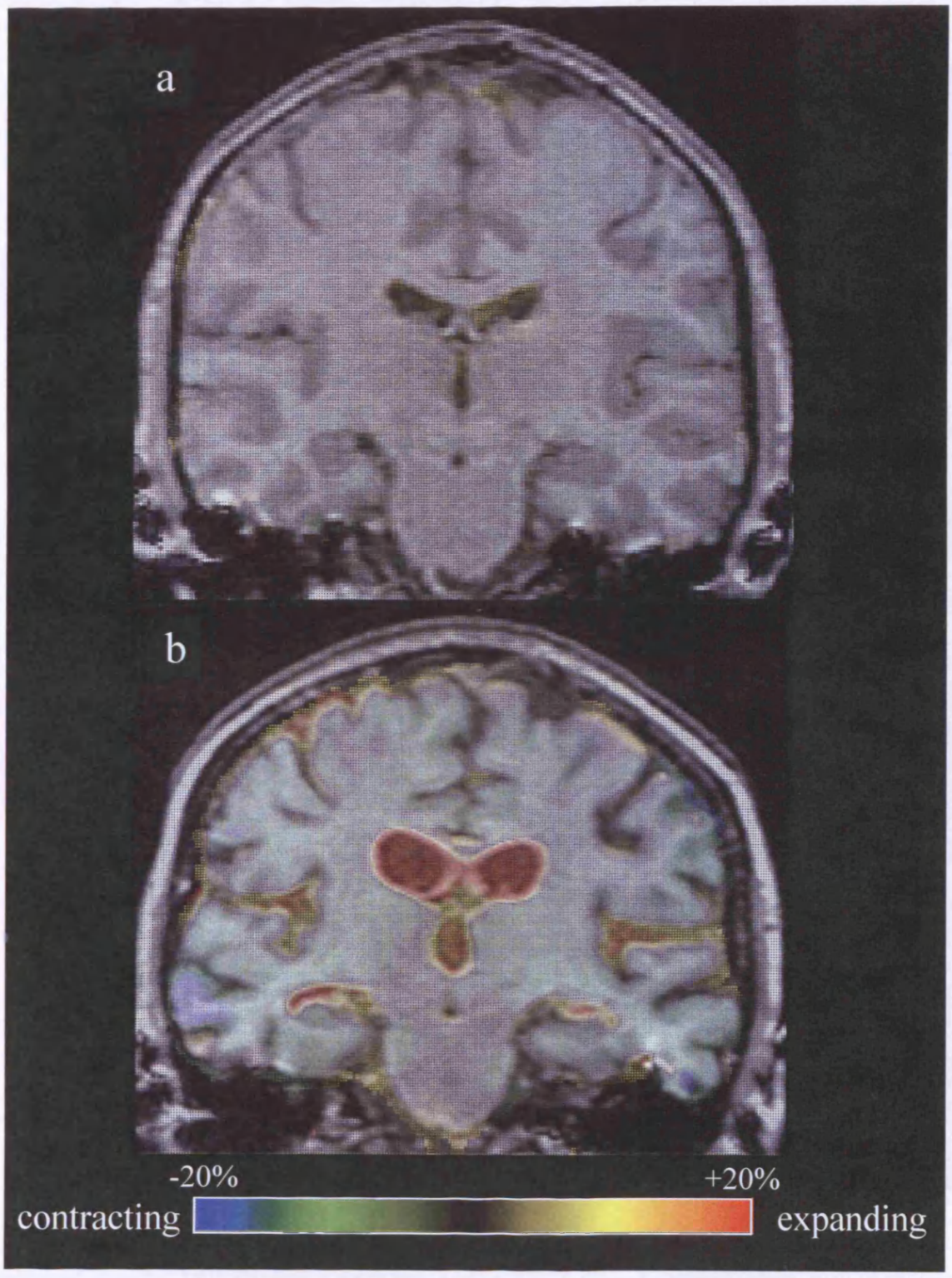
Figure 8.1 Coronal MRI scans and subtraction images in familial AD



Images of 54 year old woman with FAD

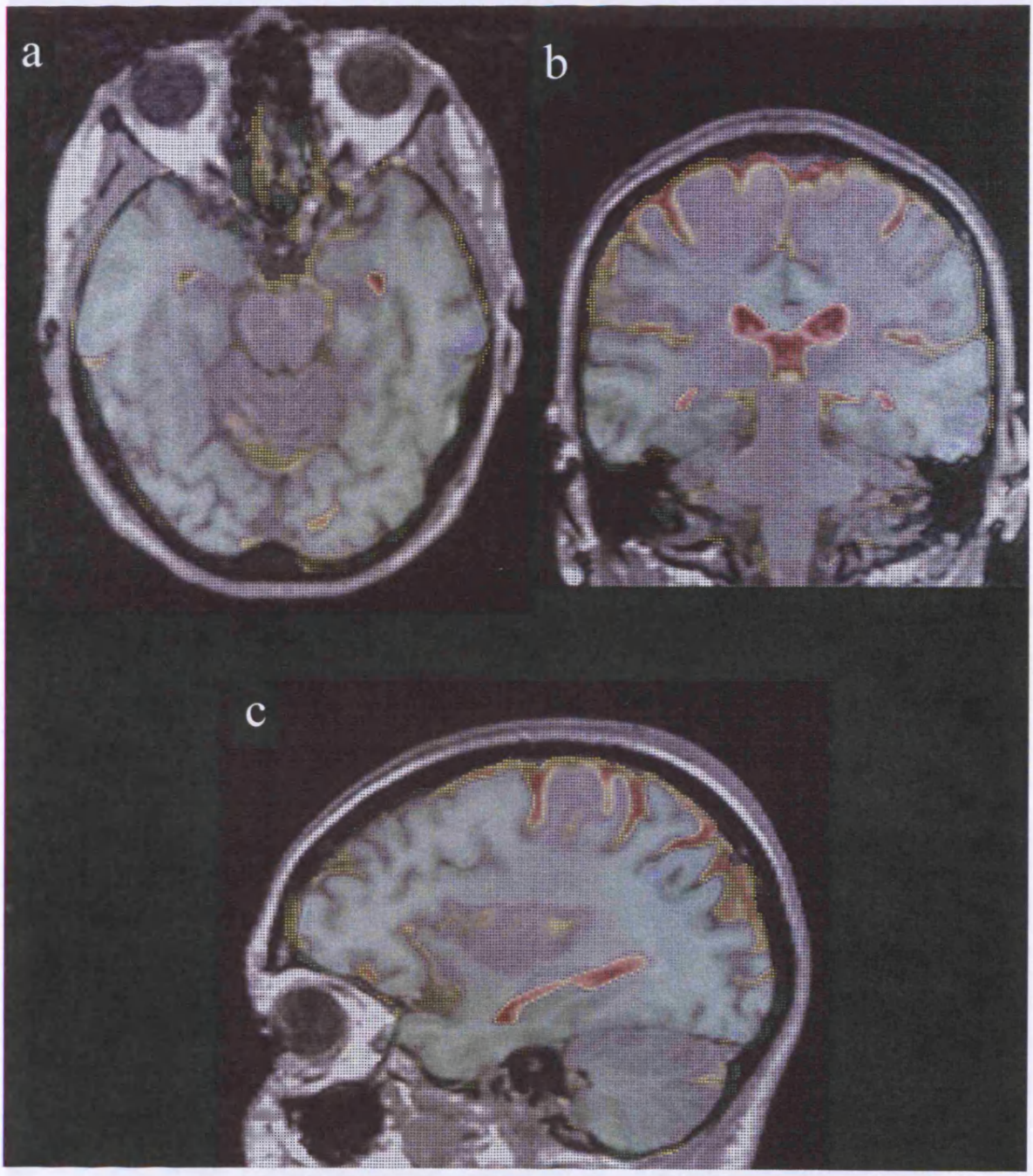
a)=Baseline MRI. b)=Repeat MRI after 14 month interval. c)=Subtraction image after rigid-body registration of the repeat scan onto the baseline image. Well matched areas appear grey while points of difference between the two scans are lighter or darker (e.g. ventricular enlargement is shown by a rim of high signal).d)=Subtraction image following fluid registration showing very good image matching.

Figure 8.2 Coronal MRIs with voxel-compression-mapping colour overlay



a)=48 year old male control with voxel compression mapping (VCM) colour overlay showing change over a period of 11 months. 2b)=46 year old female with FAD with VCM colour overlay showing change over a 14 month period.

Figure 8.3 MRI with voxel-compression-mapping colour overlay in FAD over a 3-year period during which symptoms developed



51 year old familial AD case with a 3 year interval during which time symptoms developed. VCM colour overlay in the a) axial b) coronal and c) sagittal views.

Figure 8.4 Coronal MRI slices showing VCM colour overlay in a 38 year old female familial AD case at symptom onset, but prior to clinical diagnosis.

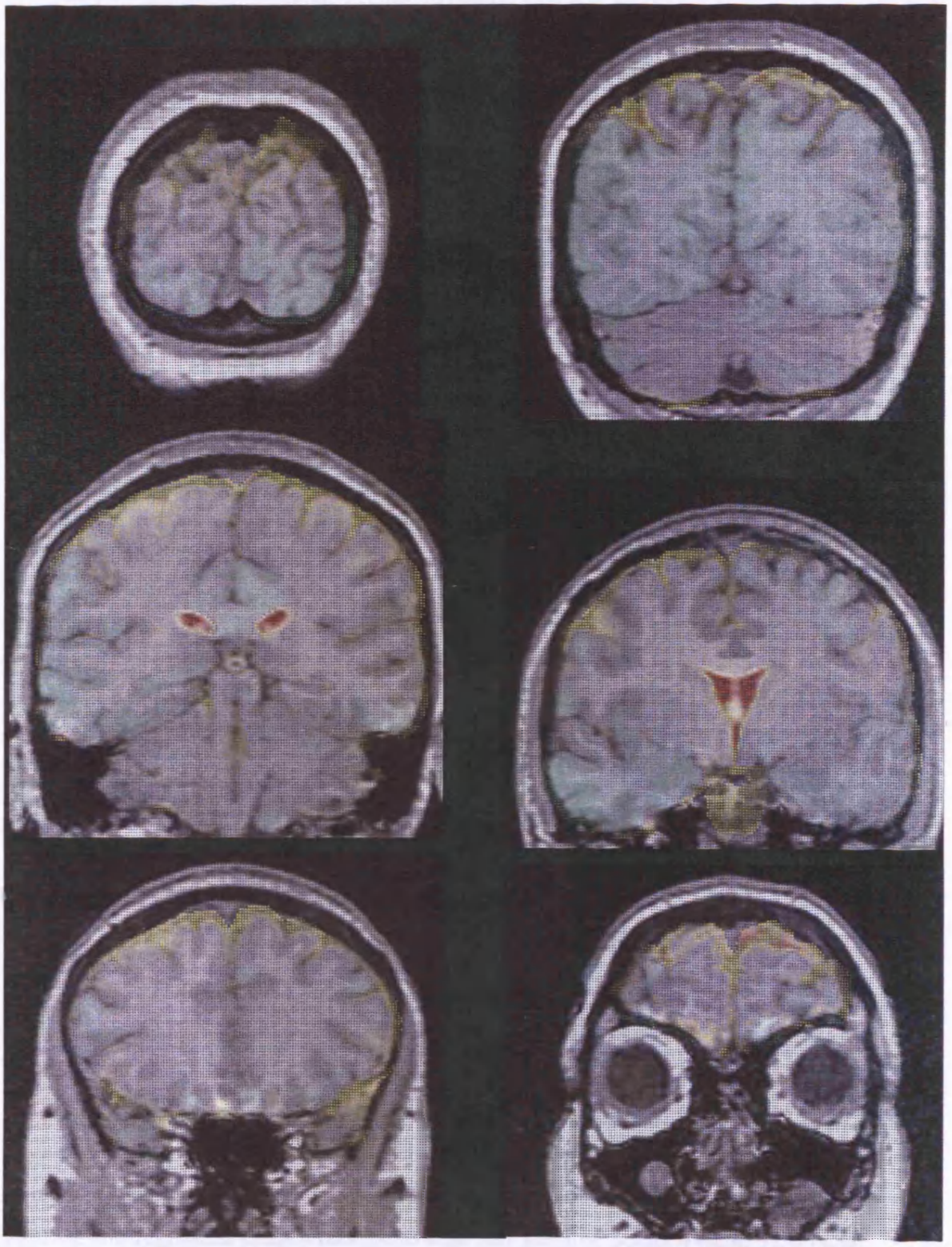
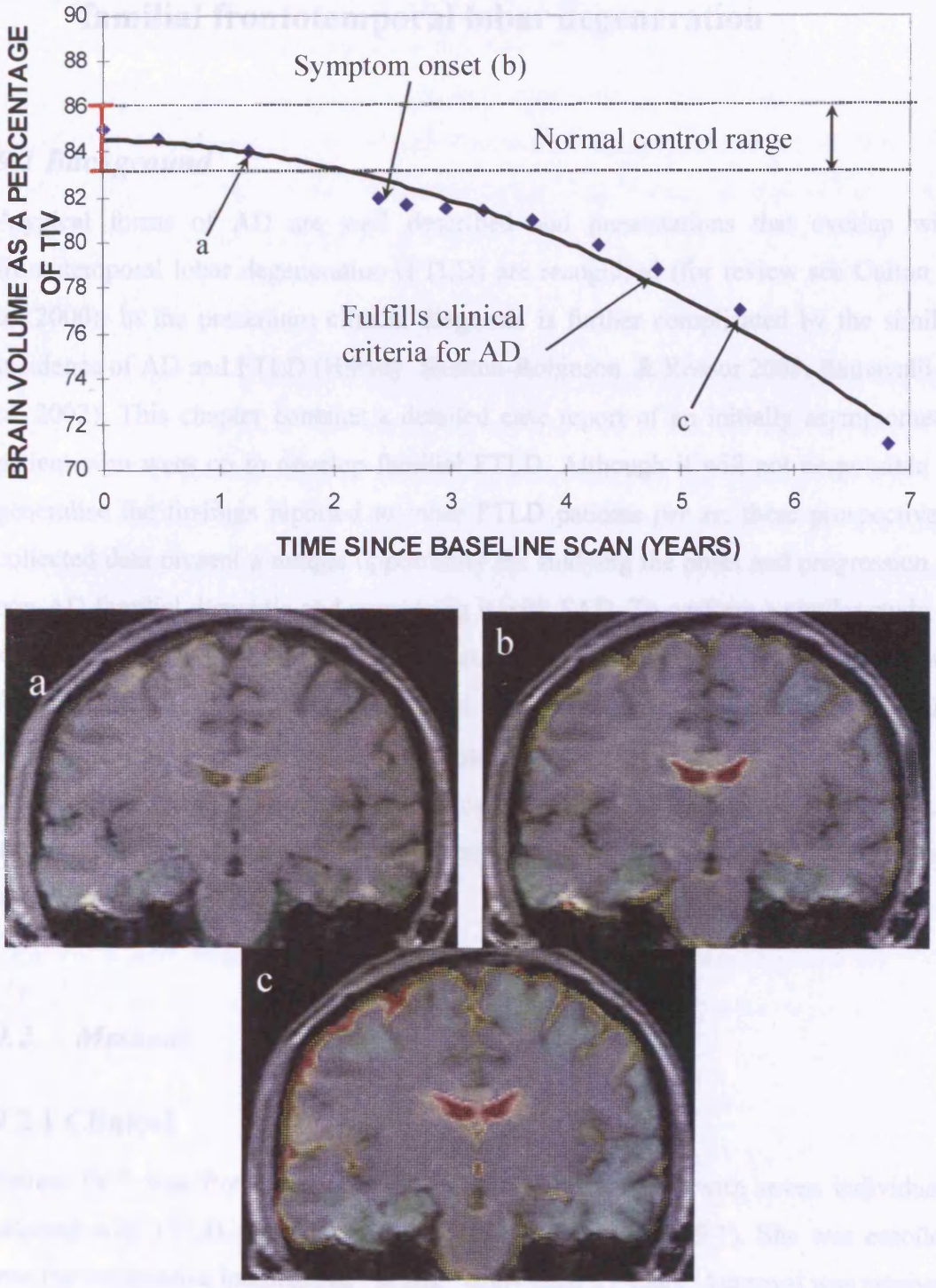


Figure 8.5 Change in brain volume in FAD



Legend: Change in brain volume as a percentage of intracranial volume over time in a familial AD patient, who was 36 years old at baseline (for normal range see Whitwell 2001). Fluid registered images relative to base line for time points *a* (scan 3), *b* (scan 4), and *c* (scan 11) are shown in the lower panel.

9 Mapping the onset and progression of atrophy in familial frontotemporal lobar degeneration

9.1 Background

Atypical forms of AD are well described and presentations that overlap with frontotemporal lobar degeneration (FTLD) are recognised (for review see Galton *et al.* 2000). In the presenium clinical diagnosis is further complicated by the similar incidence of AD and FTLD (Harvey, Skelton-Robinson, & Rossor 2003; Ratnavalli *et al.* 2002). This chapter contains a detailed case report of an initially asymptomatic patient who went on to develop familial FTLD. Although it will not be possible to generalise the findings reported to other FTLD patients *per se*, these prospectively collected data present a unique opportunity for studying the onset and progression of non-AD familial dementia and comparing it with FAD. To perform a similar study in sporadic FTLD patients would be difficult in the absence of known risk factors. Furthermore, this study permits additional assessment of the biological validity of the non-linear serial MRI registration technique (fluid registration).

The clinical and neuropsychological features of a patient with familial FTLD are correlated with the anatomical changes (as defined by means of fluid registration) and compared with FAD.

9.2 Methods

9.2.1 Clinical

Patient IV.3 was from DRG family 306, a British kindred with seven individuals affected with FTLD spanning three generations (see Figure 9.1). She was enrolled into the prospective longitudinal "at risk" study aged 51 years. Approval was received from the ethics committees of both the National Hospital for Neurology and Neurosurgery, and St. Mary's Hospital, and the patient gave informed, written consent. Assessments were conducted at approximately yearly intervals, at the Dementia Research Group and Department of Neuropsychology at the National Hospital.

Each assessment on patient IV.3 was standardised as described in chapter 2, including clinical interview, neurological examination, volumetric brain MRI, MMSE and neuropsychological assessment as described in Chapter 2.

Frozen blood for DNA extraction was only available from two family members and therefore formal linkage studies were not possible. Genomic screening for *tau* (MAPT) mutations was performed by Dr Huw Morris, Institute of Neurology, as previously described (Morris *et al.* 1999).

9.2.2 Neuroimaging

Seven T1-weighted volumetric MRI scans were acquired over a period of 68 months, using two scanners (the first three scans were performed on one scanner; the subsequent four scans on another). Both scanners were 1.5T GE Signa Units (General Electric, Milwaukee, WI). Scans were acquired using a spoiled gradient-echo technique and transferred to a Sun Enterprise 250 workstation as previously described. Each MRI scan was reported by an experienced neuroradiologist. Image processing was performed using the MIDAS software tool (Freeborough, Fox, & Kitney 1997). A semi-automated technique, using intensity thresholding and a series of erosions and dilations, was applied to delineate brain tissue. Total intracranial volumes (TIV) were measured for each scan (Whitwell *et al.* 2001). A rigid-body 9 degrees of freedom registration was performed to align the repeat scan onto the baseline image (Freeborough, Woods, & Fox 1996). Brain volumes for each scan were calculated directly from the MIDAS software tool, and corrected for TIV. Whole brain atrophy rates were calculated between intervals from the registered scans using a validated automated technique, the brain boundary shift integral (BBSI) (Fox & Freeborough 1997; Freeborough & Fox 1997). The previously described fluid registration model uses the rigidly-aligned repeat scan as a starting point to generate a voxel-level deformation field (Crum, Scahill, & Fox 2001; Freeborough & Fox 1998). The determinant of the Jacobian matrix from the deformation field gives a measure of volume change at the voxel level, and voxel compression maps are created by using a colour overlay to signify this volume change.

9.2.3 Neuropathology

Neuropathological examination was performed on patient IV.1 by Professor Lantos of the MRC Neurodegenerative Diseases Brain Bank at King's College Hospital. Further

immunohistological studies were performed in 2002/3 by Dr Keith Josephs and Dr Tamas Revesz at the Institute of Neurology. Blocks of tissue were taken from the frontal, temporal (with the hippocampus and the amygdala), parietal and occipital lobes, the basal ganglia (to include the caudate nucleus, the lentiform nucleus, the claustrum and the nucleus basalis of Meynert), the thalamus with the subthalamic nucleus, midbrain, pons, medulla oblongata and from the cerebellar hemisphere and vermis. The sections were stained with haematoxylin and eosin, impregnated with silver according to the modified Bielschowsky method and luxol fast blue and cresyl violet (on selected sections). Antibodies were used to immunostain β-amyloid, glial fibrillary acidic protein (GFAP), tau protein and α-synuclein (all from DAKO).

9.3 Results

9.3.1 Family overview

The family tree is shown in Figure 9.1. No information is available about the founders of this family. Patients II.1 and II.7 are thought to have been affected, but no further details are available. Patient III.1 died aged 57 years of lobar pneumonia, with a diagnosis of pre-senile dementia. Patient III.2 died at age 60 years of "cerebral sclerosis" in a psychiatric hospital. Patient III.4 died of bronchopneumonia, with an underlying diagnosis of pre-senile dementia, in the same hospital at age 58 years. No psychiatric in-patient notes survive for either of these two family members.

Patient IV.1 was assessed at The National Hospital for Neurology when he presented aged 56 years with a four year history of personality change, disordered social conduct and cognitive decline. Four years after onset, he was wheelchair bound and incontinent, laughed in response to questioning or any attempt to engage him in conversation, and had no comprehensible speech. Brain CT revealed generalized involutional changes particularly prominent over the frontal and temporal areas. He died twelve months later and underwent neuropathological examination.

The genomic screen for *tau* (MAPT) mutations performed on patients IV.1 and IV.3 was negative.

9.3.2 Neuropathology

The brain of patient IV.1 was severely atrophic with the left frontal and temporal lobes most affected. Microscopy revealed no plaques or tangles but showed neuronal loss and astrocytosis with status spongiosus in the superficial layers of the temporal and especially frontal cortex. Glial fibrillary acidic protein immunocytochemistry confirmed the increase in astrocytes in both cortex and underlying white matter. Immunohistochemistry for ubiquitin revealed a small number of intracytoplasmic inclusions in the hippocampal dentate granule cells. A moderate number of abnormal neurites and intracytoplasmic inclusions were noted in the superficial layer of the frontal and temporal lobe cortices as well as a moderate number of intranuclear inclusions in the limbic cortices. Tau and α-synuclein immunohistochemistry was negative. The postmortem diagnosis was of a familial FTLD with ubiquitin positive (tau and synuclein negative) inclusions.

9.3.3 Prospective study of patient IV.3

During the period of study patient IV.3 attended for nine clinical visits. Neuropsychological assessments were performed at six of these time-points, and MRI was performed at seven. Details of these assessments at each time point are presented below, with an overview shown in Figure 9.2. The neuropsychological scores are summarized in Table 9.1.

At the commencement of the study (visit 1) patient IV.3 was 51 years old, and had been working as a librarian for 26 years, having initially trained as a nurse. She was left-handed. She did not smoke and only rarely drank alcohol. She reported no cognitive or behavioural symptoms, and the clinical assessment was unremarkable, with no objective evidence of cognitive impairment. She scored 29 on the MMSE.

At neuropsychological assessment (1), on the shortened version of the WAIS-R, she scored at an average level on the Verbal scale (IQ 97), and at the upper end of the low average range on the Performance scale (IQ 87). She performed at an average level on the Advanced Progressive Matrices, a test of nonverbal abstract reasoning (Raven 1965). Her premorbid optimal level of functioning was estimated using the NART to

be in the superior range (123) (Nelson & Willison 1991). There was a significant discrepancy between the estimated premorbid and current performance on tests of general intelligence. This indicates a significant intellectual decline (Nelson & O'Connell 1978). There was however no evidence for deficits in memory, visuoperceptual or frontal executive function.

Cross-sectional MRI (1) was reported as normal; however the initial calculated brain volume was slightly small at 1099ml or 82% of total intracranial volume (normal range: 83-89%).

Visit 2 (14 months)

At the second assessment, she reported no cognitive or behavioural changes. The clinical assessment was unremarkable; she scored 28 on the MMSE. There was no evidence for decline on neuropsychological testing (2); she scored at a slightly higher level on both the verbal and performance sections of the WAIS-R. Her performance on the Advanced Progressive Matrices remained in the average range (Raven 1965). All other test scores were essentially unchanged.

The MRI (2) was reported as normal.

Applying fluid–registration to MRIs (1) and (2) revealed very focal left sided frontal atrophy almost completely confined to the *pars opercularis* occurring over the first 14 month period. (Figure 9.3).

Visit 3 (26 months)

At the third assessment the patient remained well and she reported no cognitve or behavioural changes. Clinical assessment was normal and she scored 29 on the MMSE.

The third neuropsychological assessment revealed that her verbal and performance IQs remained static at an average level. Despite intact naming skills she performed remarkably poorly on a verbal phonemic fluency task. However, her performance remained unimpaired on three further frontal tasks (Wisconsin card sorting test, Cognitive Estimates test, Trail Making test). Performance on other focal cognitive tasks including memory remained unchanged.

The MRI (3) was reported as normal. Using rigid body registration and the brain boundary shift integral (BBSI) we demonstrated that over the time period

spanning the first three assessments there was 22.9ml brain volume loss (assuming constant brain loss this equates to 0.96% of brain volume/year).

Fluid-registration was applied to MRIs (2) and (3). Over this 12 month period diffuse left sided frontal lobe atrophy had developed, though the *pars opercularis* was still losing volume at the greatest rate. There was now clear, but lesser involvement of the *pars triangularis*, the *pars orbitalis* and the middle frontal gyrus. The anterior caudate was slightly involved, but the primary motor cortex, paracentral lobule, temporal lobe structures (including the hippocampi) and cerebellum remained unaffected (see Figure 9.4 and 9.5).

Visit 4 (45 months)

At the fourth assessment 19 months later, for the first time the patient reported symptoms. She was having difficulties expressing herself, and felt that her childhood stammer had re-emerged, although this was not apparent during the interview. She reported some difficulties with mental arithmetic. She denied any difficulties learning or speaking her lines when acting, or any difficulties at work. The abnormalities in the clinical assessment were confined to higher mental function; throughout the interview she appeared more jocular than previously, and laughed inappropriately on occasion. She scored 29 on the MMSE, although she was no longer able to serially subtract 7s, and achieved this score by spelling "WORLD" backwards.

Neuropsychological testing (4) revealed that her scores remained within the average range in the verbal and performance part of the WAIS-R, and in the Ravens Advanced Matrices. Her spelling had declined slightly from the high average/superior to the average level, and arithmetic was now in the low-average range. Nonetheless, memory, naming, speed and attention, visual perceptual and visuospatial skills were unchanged. She obtained an impaired score on a test of frontal lobe function (the Hayling sentence completion test). Despite good scores on tests of speed and attention her performance on the Trail Making Test was slow.

For the first time her volumetric MRI (4) was abnormal. It was reported as showing enlarged lateral ventricles and widening of the Sylvian fissure on the left.

Visit 5 (51 months)

By the time of the fifth assessment, six months later, the patient reported significant deterioration. She now had clear difficulties with expressive language, had stopped

using the telephone, and could no longer work. She reported that she had become "much quieter" at social occasions. She was remarkably unconcerned by her symptoms, and by the fact that she had been referred from her work for an occupational health assessment. The clinical examination was now clearly abnormal. She had speech production difficulties and made several phonemic errors in both spontaneous speech, and when reading a prose passage. She laughed inappropriately on several occasions, and manifested bilateral limb dyspraxia, most marked when miming meaningless gestures with the right hand. She had orofacial dyspraxia; she could neither whistle nor cough; when asked to yawn she repeated the word "yawn". She was unable to perform bimanual movements, an alternating coin test, nor a conflicting tapping test. She scored 26 on the MMSE.

Neuropsychological testing (5) revealed a decline in her verbal and performance IQ. Her memory and naming continued to be unaffected, although her single word comprehension had declined slightly to the average level. Her spelling had declined further to the low end of the average range. Calculation was now defective. There was impairment on the Cube Analysis subtest of visuospatial function, although visual perceptual skills were normal. There was evidence of further frontal executive impairment, with decline on the Wisconsin card sorting and Trail Making tests. Her performance on tests of speed and attention remained normal.

Standard MRI (5) demonstrated further cerebral atrophy, with increasingly prominent lateral ventricles and widening of the left peri-sylvian fissure.

Visit 6 (56 months)

At the sixth assessment she was seen at the Specialist Cognitive Disorders Clinic. She had previously been keen to attend alone not wanting to burden her husband with taking part in a research project. Her husband was therefore for the first time able to provide a corroborative history. He had been aware of his wife's problems expressing herself verbally for about a year. Her childhood stammer had reappeared and her arithmetic had declined. She had become less tidy and on occasions had left the kitchen tap running. On no occasion had she got lost. Her husband also reported that she laughed inappropriately. There was no change in religiosity nor libido. Her food preferences had changed, e.g. she no longer liked potatoes. Speech production difficulties were evident. Her spontaneous speech was characterised by phonemic

errors and echolalia. There was evidence of orofacial apraxia, and she failed bedside tests sensitive to frontal lobe dysfunction. She scored 26/30 on the MMSE.

Neuropsychological assessment (6) revealed further decline in verbal and performance IQ to a borderline defective level. For the first time there was evidence of language and verbal memory impairment. Verbal recognition memory scores had declined to a defective level and her verbal recall memory to a low average level. In contrast visual memory remained at a good average level. She failed to score on tests of calculation and spelling. She was almost unable to score on a test of visuospatial skills. Her visuoperceptual skills had declined to a low average level. She failed all frontal executive tests, with the exception of the Weigl colour form sorting test. Her performance on two tests of speed and attention remained within normal limits. Subsequently she was no longer able to tolerate formal neuropsychological testing.
The MRI scan (6) was clearly abnormal with prominent ventricles, and asymmetric left temporal lobe atrophy. Rigid body registration and calculation using the BBSI, revealed that over the eleven month interval between scans four and six, 43.8ml of brain volume was lost (i.e. 4.5% of brain volume/year, assuming linear decline).

Fluid-registration of MRIs (4) and (6) revealed increasing atrophy particularly involving the left orbito-frontal region and right anterior frontal lobe, including the operculum over the 11 month period. There was more widespread atrophy diffusely involving the left temporal lobe, with the inferior temporal lobe gyrus, and to a lesser extent the middle temporal gyrus being affected. There was also involvement of the left parietal lobe and both frontal lobes, with relative sparing of the orbito-frontal gyri. The primary sensory and motor cortices, superior temporal gyri, hippocampi, amygdalae and cerebellum remained unaffected (see Figure 9.6, 9.7 and 9.8).

Visit 7 (57 months)

One month later the patient was admitted for investigation at the National Hospital for Neurology and Neurosurgery the following month, by which time she had gained weight and had developed a clear, new preference for sweet foods. Clinical examination revealed her to be perseverative, and to exhibit utilization behaviour and bilateral grasp reflexes; there were no fasciculations, and the remainder of the examination was unchanged from her previous assessment. The MMSE was now 21/30. Screening blood tests for reversible causes of dementia were normal or

negative, including electrolytes, glucose, renal function, liver function, thyroid function, full blood count, erythrocyte sedimentation rate, clotting studies, vitamin B12, folate, an autoantibody screen, and treponemal serology. The CSF was acellular, the protein and glucose were normal, and there were no oligoclonal bands in CSF or serum. The EEG was normal with preservation of α rhythm. Neuropsychological testing was not repeated. She was diagnosed with familial FTLD.

Visit 8 (66 months)

By this stage the patient was practically mute, and could no longer cope with the demands of formal neuropsychological assessment. She occasionally said "yes" when asked a question, but not always appropriately. She was no longer able to shop by herself, but did wander without getting lost.

Visit 9 (69 months)

At this final assessment, the only vocalization was a giggle. She had deteriorated significantly and was now occasionally incontinent of urine. She no longer recognized her friends. Her sense of humour had become puerile, but she continued to enjoy simple comedy shows on television. She now had severe oro-facial apraxia which interfered with mastication.

The cross-sectional MRI (7) revealed further asymmetric left temporal atrophy. However, despite very disabling symptoms, there was sparing of the hippocampi, mesial temporal lobe, anterior superior temporal gyri, occipital poles and cerebellum, and even at this stage frontal lobe atrophy was not prominent. Using rigid body registration and the BBSI to compare scans six and seven (an interval of thirteen months) 76.3ml of brain volume was lost (8% of brain volume/year).

9.4 Discussion

This study reports the clinical, neuropsychological and imaging findings of a patient with familial FTLD over the time period she progressed from being asymptomatic to being profoundly affected. Fluid registration demonstrates early left-sided focal brain atrophy with subsequent progressive clinical and neuropsychological decline. It seems reasonable to assume that this focal atrophy is responsible for the observed deficits, and that these changes represent early manifestations of the disease process, years

before the disease would have presented clinically had she not been followed on the basis of her family history. In addition, the focal left sided atrophy with associated speech production deficits is compelling evidence that our patient, despite being left-handed, was left hemisphere dominant.

Arnold Pick first described focal brain atrophy in 1892 (Pick 1892). Since that time multiple pathological studies have shown that degenerative dementias may be associated with relatively focal, lobar atrophy. Neuroimaging studies have allowed these changes to be delineated *in vivo*, and correlated with concomitant cognitive deficits. Magnetic resonance imaging has been used to demonstrate strikingly different patterns of regional atrophy within the subgroups of FTLD (Chan *et al.* 2001b; Perry & Hodges 2000). However, such cross-sectional studies cannot demonstrate the progression of atrophy, one of the hallmarks of a neurodegenerative disease. Analysis of serial scans from the same patient can provide an accurate measure of change within an individual over time. Such methodologies have been used to demonstrate significantly increased rates of global atrophy in individuals with AD and FTLD (Chan *et al.* 2001a; Fox, Freeborough, & Rossor 1996) when compared with control subjects. In this study excess global atrophy was occurring from the start of the study, and accelerated during the course of the disease in line with both the clinical and neuropsychological progression.

The development of fluid registration allows visualization of the progression of regional brain atrophy *in vivo*. This technique has been validated in both AD and control subjects (Freeborough & Fox 1998).

At this patient's first visit, despite the reported lack of symptoms or abnormal clinical findings, it is notable that neuropsychological testing revealed significant intellectual impairment in the setting of well-preserved cognitive skills. This finding indicates that intellectual impairment was present even at this very early stage. Recent studies have shown that the performance on tests of general intelligence, in particular tests of fluid intelligence such as the Advanced Matrices, is related to brain function in the lateral prefrontal cortex (Gray, Chabris, & Braver 2003). Thus patient IV.3's impaired performance on tests of general intelligence may suggest early frontal dysfunction. However, Geschwind *et al.* have shown that frontal executive impairment may be present in patients destined to get FTLD decades before the onset of symptoms (Geschwind *et al.* 2001), and in patient IV.3 there was evidence of further neuropsychological decline only at the third assessment. It is therefore is

unclear whether the documented intellectual impairment represented the onset of the disease process, or was longstanding.

Fluid-registration of the first and second MRI scans revealed highly selective left sided frontal atrophy almost completely confined to the *pars opercularis*. This change was accompanied neither by clinical nor neuropsychological evidence of decline. By the third scan this atrophy pattern had progressed and extended to involve the *pars triangularis*, the *pars orbitalis*, and, to a lesser extent, the middle frontal gyrus. This increased, but still focal, atrophy was not accompanied by clinical evidence of decline. However, neuropsychological testing revealed consistent evidence of a new selective verbal (phonemic) fluency deficit. Since Broca's first descriptions of focal dominant frontal lesions causing expressive language dysfunction, numerous neuropsychological studies have demonstrated that the left frontal lobe is implicated in the organisation and generation of verbal output (Milner 1982; Robinson, Blair, & Cipolotti 1998; Troyer, Moscovitch, & Winocur 1997). A recent review of the literature has proposed that lesions of the dominant *pars opercularis* may impair the ability to sequence words syntactically, and that lesions of the *pars triangularis* may impair the ability to generate words (Taubner, Raymer, & Heilman 1999). Positron emission tomography studies have also shown activation of left dorsolateral prefrontal cortex, Broca's area and anterior cingulate during word generation tasks (for a review see Nathaniel-James & Frith 2002). In conclusion it is likely that the demonstrated atrophy pattern is responsible for the observed impairment in verbal fluency, but that there was evidence of very focal atrophy in this region that preceded any clinical or neuropsychological evidence of progressive impairment.

The reported findings findings are broadly in keeping with previous functional neuroimaging studies of speech production in normal volunteers (Blank *et al.* 2002) and after stroke (Blank *et al.* 2003). However, relatively focal cerebral hypometabolism was demonstrated in the left anterior insula in a group study comparing PA and AD patients (Nestor *et al.* 2003). Since the PA patients already fulfilled diagnostic criteria, by definition the demonstrated focal metabolism was later in the disease process than the presymptomatic focal left opercular atrophy in our patient. Furthermore this difference may be contributed to by the heterogeneity of age, disease duration and possibly the underlying neuropathophysiology of the PA

patient group. So although these studies are clearly complementary they are not directly comparable.

The subsequent course of this patient's disease was characterized clinically by a progressive decline in speech production, and the emergence of increasing frontal executive and dominant parietal dysfunction. These findings were confirmed on neuropsychological testing. At visit 4, impairment on the Hayling sentence completion test and probable decline on the Trail Making test were documented, with subsequent failure on the Wisconsin card-sorting and Trail Making tests at assessment 5 illustrating clear progression of frontal dysexecutive impairment. Increasing deterioration of calculation and spelling was confirmed over visits 4-5, consistent with increasing dysfunction of the left parietal lobe. By the final neuropsychological assessment (6), there was further significant deterioration of intellectual function, with by now marked global impairment of executive function and severe dyscalculia and dysgraphia. Speech production difficulties were by now prominent. At this stage, performance on a demanding series of memory tests remained broadly within normal limits. Similarly, her naming, verbal comprehension and speed and attention were satisfactory.

Fluid registration over the time period of scans (4)-(6) revealed increasing atrophy centred around the left frontal and left parietal lobes, findings consistent with those from clinical assessment and neuropsychological testing. No significant atrophy was seen in the hippocampi and medial temporal lobes, areas implicated as neuronal substrata of verbal and visual memory function (Cipolotti *et al.* 2001; Milner 1966), compatible with the patient's well-preserved memory. The re-emergence of primitive reflexes, generally implicated in frontal lobe disease, in the absence of long-tract or cerebellar signs is also compatible with the observed atrophy pattern. The increasing rates of global brain atrophy over the period of follow-up are in keeping both with the increasing regional atrophy demonstrated using fluid registration, and the progression of the disease as assessed by clinical and neuropsychological testing. Importantly, there were no abnormalities visible on cross-sectional imaging until scan (4), 45 months into the study, and 31 months after clear focal changes had been demonstrated using fluid-registration. When abnormalities did become present on conventional imaging, they were entirely in keeping with a diagnosis of FTLD.

This study has a number of limitations. The patient attended alone (at her request) until the 6th assessment, and the history given may therefore be incomplete.

However, when she was accompanied by her husband, his account of her illness was broadly in keeping with our clinical assessment. We have no information concerning the early course of the disease in other affected family members. This patient's brother was not assessed until late in his disease, at which time many of the reported clinical features appear to be consistent with FTLD. Given this, the family history, and similar young age at onset, it is highly likely that despite differing phenotypes, both siblings were suffering from the same disease process. A recent study suggested that in FTLD, ubiquitin positive intranuclear inclusions are specific for familial FTLD with ubiquitin inclusions, and may even identify a subset of families with a common molecular pathogenesis (Mackenzie & Feldman 2003), providing further support for these two siblings being affected by the same disease. However, as FTLD is a clinically and neuropathologically diverse entity, the pattern and progression of atrophy we describe in this individual are unlikely to be consistent amongst different clinical presentations, nor necessarily applicable to sporadic FTLD.

Though the use of two different scanners could result in a systematic bias regarding the reported progression of atrophy, the earliest changes were demonstrated on the first scanner and no "cross scanner" calculations or analyses were performed. Furthermore all reported atrophy results are corrected for headsize (TIV) which can reasonably be assumed to remain constant.

The neuropsychological battery used in this study, although comprehensive was initially designed to detect the earliest changes in FAD, and not FTLD *per se*. Accordingly the selected tests were not specifically targeted to detect early speech production deficits. It is therefore possible that with closer assessment, subtle speech production dysfunction might have been present even earlier than was detected.

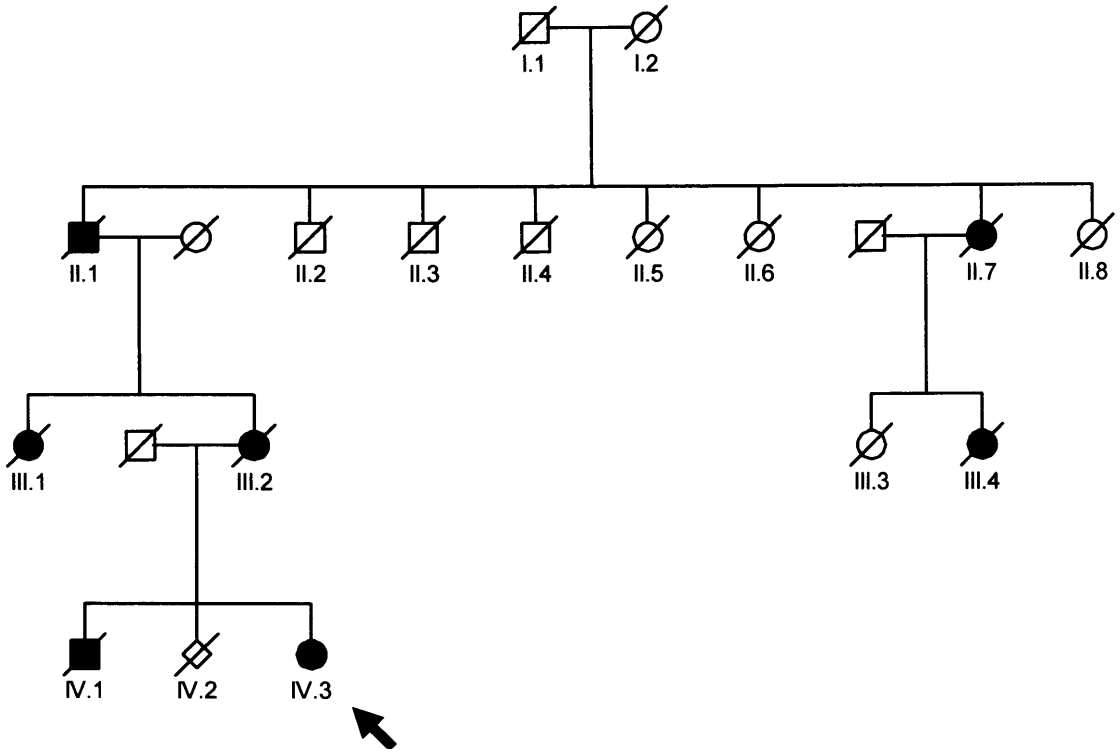
In summary the onset and progression of familial FTLD is markedly different from the findings reported in Chapter 8 for FAD with initially highly selective left sided frontal atrophy, then more prominent left sided frontal atrophy, followed by involvement of the left parietal lobe. Unlike AD no significant atrophy was seen in the hippocampi or medial temporal lobe structures. This study provides support for the biological validity of the fluid registration technique with atrophy reflecting the different clinical presentation and different pathophysiological processes.

Table 9.1 Neuropsychological assessments of patient 306.IV.3.

Assessments	1	2	3	4	5	6
	Feb 1996	May 1997	Apr 1998	Nov 1997	May 2000	Oct 2000
Month	0	14	26	45	51	56
Verbal IQ	97	108	103	97	85	75
Performance IQ	87	96	89	92	85	72
Advanced Matrices	7/12	n.t.	6/12	6/12	n.t.	n.t.
NART IQ	123	n.t.	n.t.	n.t.	n.t.	n.t.
RM Words	48/50 (>75%ile)	48/50 (>75%ile)	49/50 (=90%ile)	48/50 (75-90%ile)	42/50 (=50%ile)	33/50 (<5%ile)
RM Faces	49/50 (>75%ile)	47/50 (= 75%ile)	50/50 (>95%ile)	47/50 (= 75%ile)	39/50 (= 25%ile)	43/50 (50-75%ile)
Topographical	n.t.	n.t.	n.t.	n.t.	28/30 (95%ile)	23/30 (=50%ile)
Paired Associates 1	24/24 (>90%ile)	24/24 (>90%ile)	21/24 (= 75%ile)	19/24 (= 50%ile)	20/24 (= 75%ile)	20/24 (>75%ile)
Paired Associates 2	24/24 (>90%ile)	24/24 (>90%ile)	24/24 (>90%ile)	22/24 (= 25%ile)	24/24 (>75%ile)	18/24 (10-25%ile)
GD Naming Test	25/30 (75-90%ile)	24/30 (75-90%ile)	27/30 (=95%ile)	25/30 (75-90%ile)	24/30 (75-90%ile)	20/30 (25-50%ile)
Synonyms	47/50 (>75-	47/50 (75-	47/50 (75-90%ile)	44/50 (50-75%ile)	42/50 (=50%ile)	27/50 (1-5%ile)
GD Spelling Test	27/30 (>90%ile)	29/30 (>90%ile)	29/30 (>90%ile)	22/30 (50-75%ile)	7/30 (10-25%ile)	0/30
GD Arithmetic Test	11/24 (= 50%ile)	10/24 (25-50%ile)	10/24 (25-50%ile)	5/24 (10-25%ile)	3/24 (=5%ile)	0/24
Silhouettes	23/30 (= 50%ile)	22/30 (25-50%ile)	24/30 (50-75%ile)	24/30 (50-75%ile)	20/30 (25-50%ile)	17/30 (10-25%ile)
Cube Analysis	10/10 (>5% cut-off)	10/10 (>5% cut-off)	9/10 (>5% cut-off)	8/10 (>5% cut-off)	6/10 (<5% cut-off)	2/10 (<5% cut-off)
WCST	Pass	Pass	Pass	n.t.	Fail	n.t.
Cognitive Estimates	4	3	2	n.t	n.t.	n.t.
"S" words	n.t.	n.t.	9	8	6	1
Trail Making A	n.t.	n.t	53 (25-50%ile)	52 (25-50%ile)	76 (10-25%ile)	Unable to complete
Trail Making B	n.t.	n.t.	89 (25-50%ile)	120 (25-50%ile)	160 (=10%ile)	Failed practice
Hayling	n.t.	n.t.	n.t.	1 (fail)	3 (poor)	3 (poor)
Weigl	n.t.	n.t.	n.t.	n.t.	n.t.	Pass
Digit Copying	40 (50-75%ile)	42 (=50%ile)	39 (50-75%ile)	41 (50-75%ile)	45 (50-75%ile)	56 (10-25%ile)
Cancelling 0's (mean;sd)	67 (46;14)	64 (46;14)	48 (46;14)	51 (46;14)	64 (46;14)	51 (46;14)

Legend: RM = Recognition Memory; GD = Graded-Difficulty; WCST = Wisconsin Card Sorting Test; n.t. = not tested

Figure 9.1 Family tree of family 306



Patient enrolled in prospective longitudinal study marked with arrow. Circles represent females, squares males, and diamonds are anonymised individuals. Deceased family members are marked with a diagonal slash

Figure 9.2 Overview of assessments that patient IV.3 underwent during the course of the study

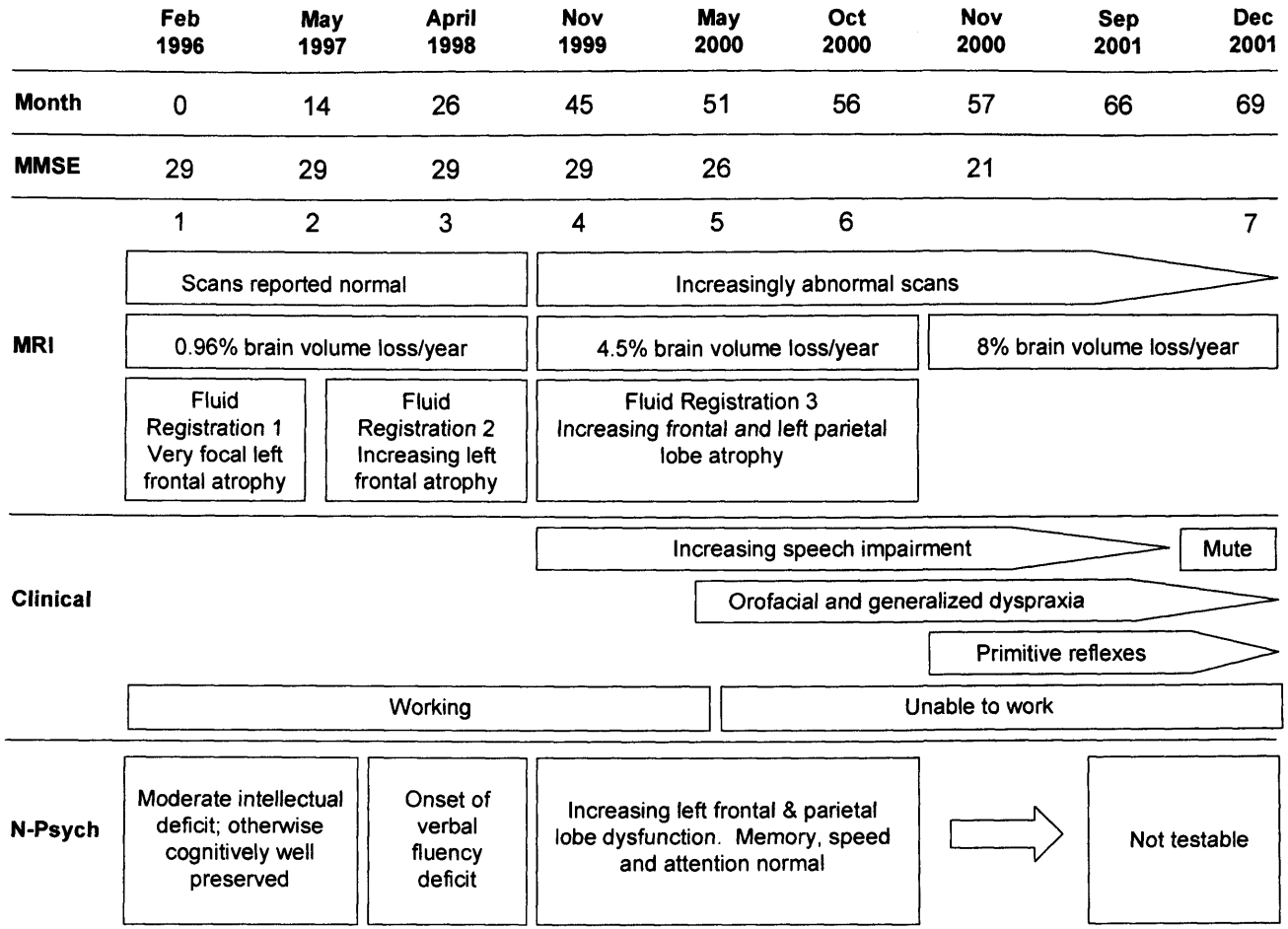
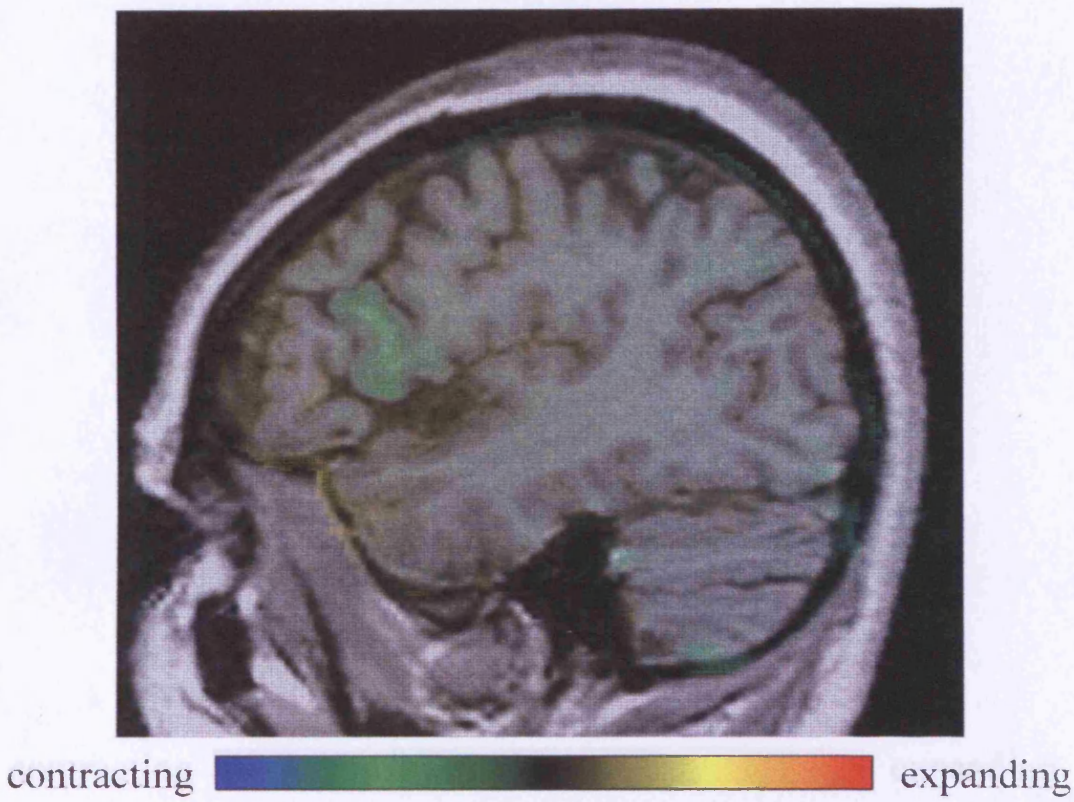
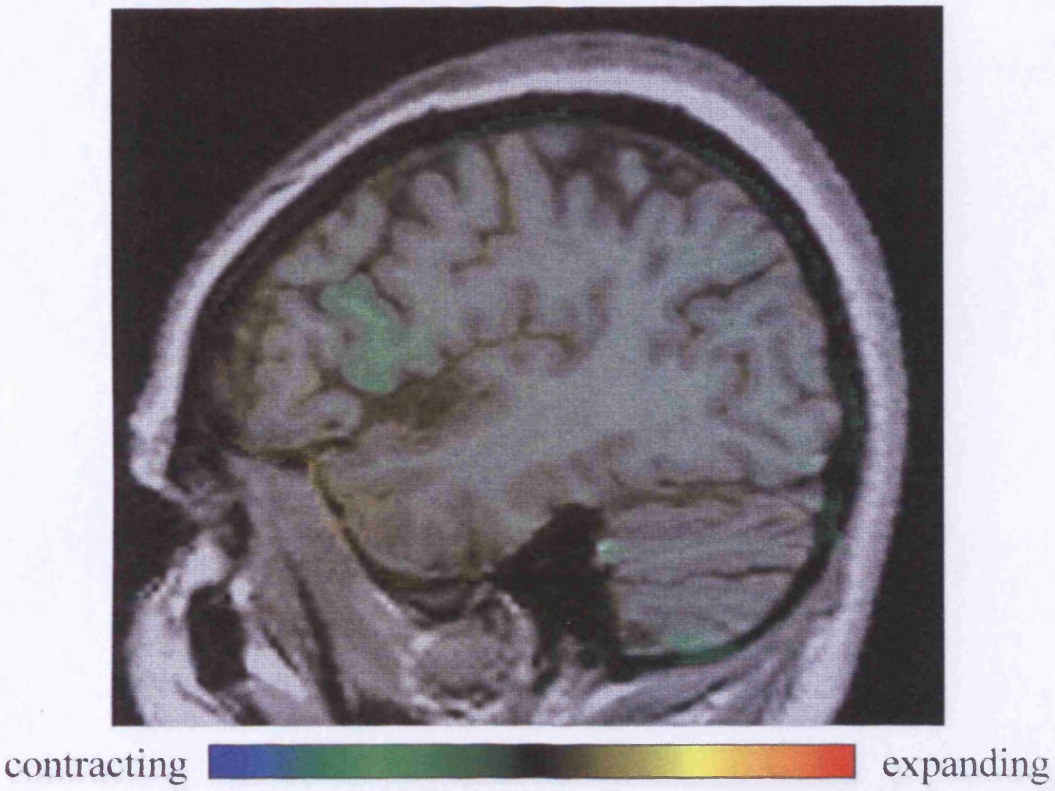


Figure 9.3 Voxel compression map time period one (sagittal)



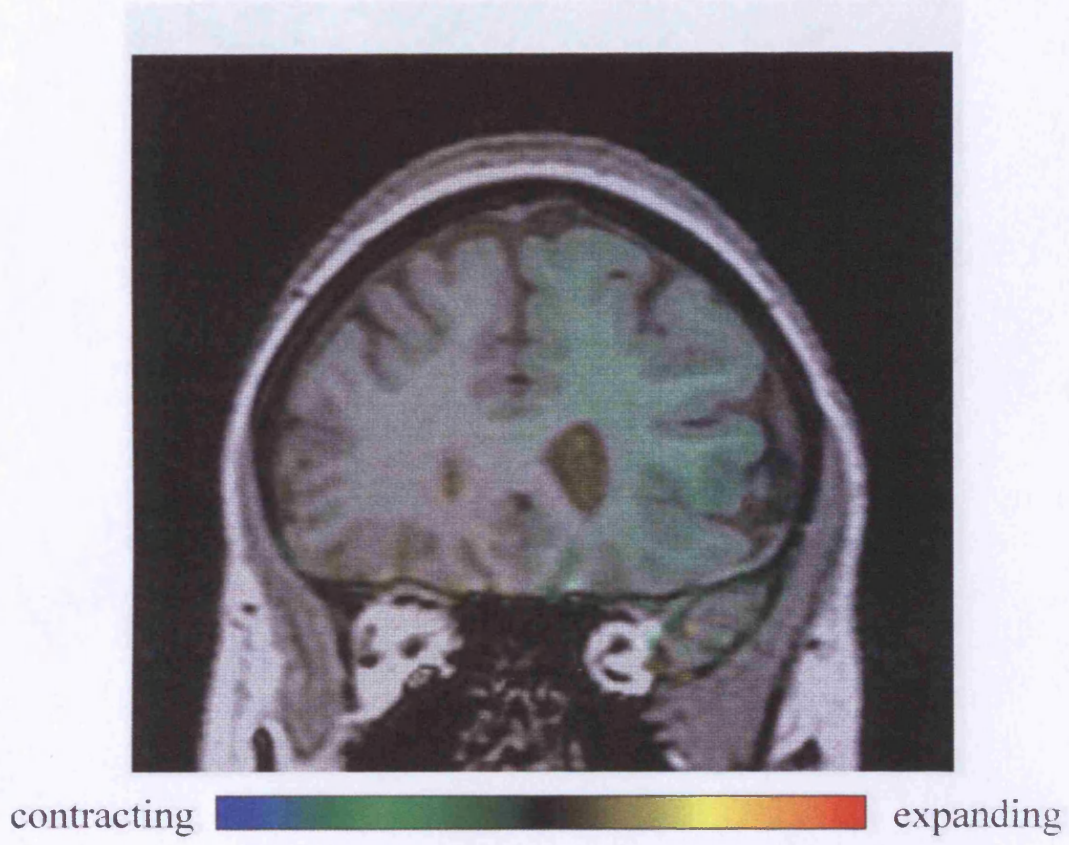
Sagittal MRI of patient 306.IV.3 with colour overlay over time period one, showing very focal anterolateral left frontal lobe atrophy, particularly centred around the pars opercularis

Figure 9.4 Voxel compression map time period two (sagittal)



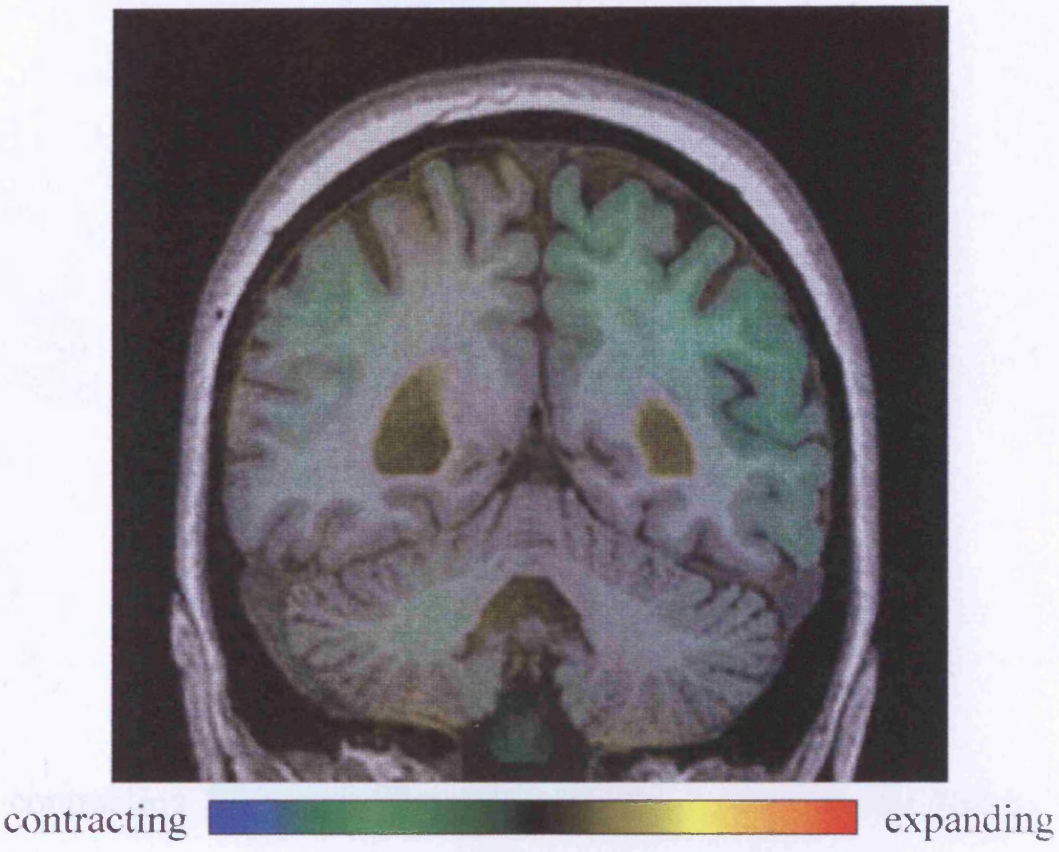
Sagittal MRI of patient 306.IV.3 with voxel-compression-mapping overlay over time period two, showing focal anterolateral left frontal lobe atrophy, extending from, but still centred around the pars opercularis.

Figure 9.5 Voxel compression map time period two (coronal)



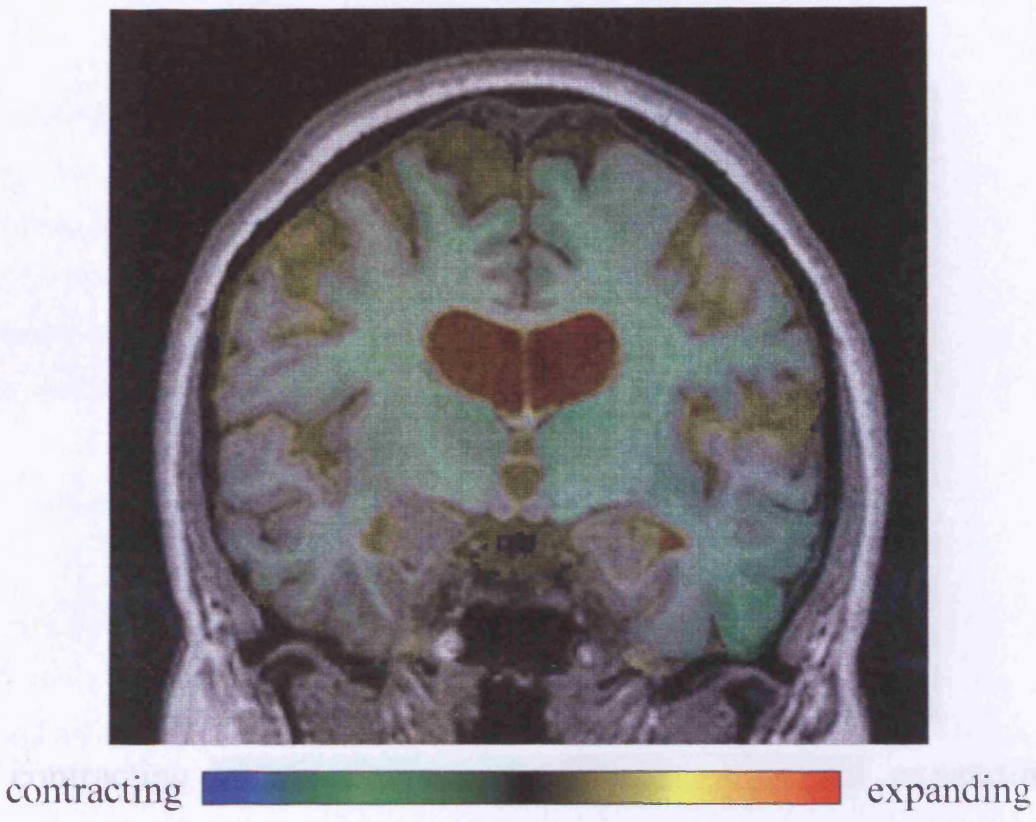
Coronal MRI of patient 306.IV.3 with voxel-compression-mapping overlay over time period two, showing marked asymmetric (Left >Right) frontal lobe atrophy at this stage

Figure 9.6 Voxel compression map time period three (coronal-parietal)



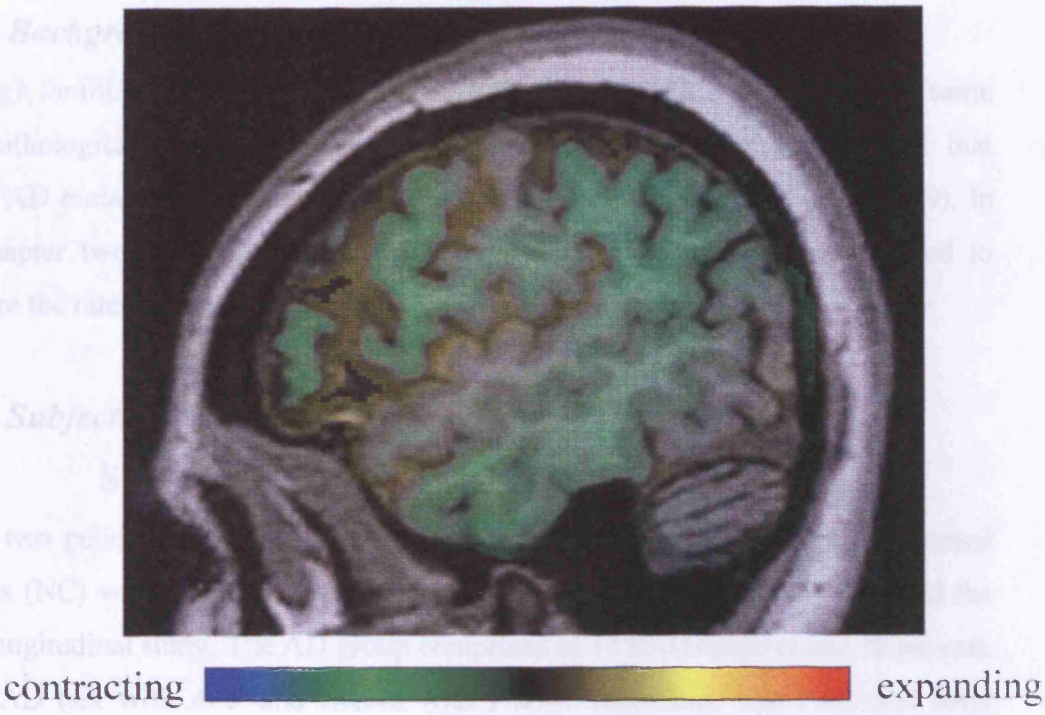
Coronal MRI of patient 306.IV.3 with voxel-compression-mapping overlay over time period three showing focal left parietal lobe atrophy. The right parietal lobe is much less affected, and with no significant change seen within the cerebellum.

Figure 9.7 Voxel compression map time period three (coronal-frontal)



Coronal MRI of patient 306.IV.3 with voxel-compression-mapping overlay over time period three, showing bilateral frontal lobe atrophy, more prominent on the left. There is focal atrophy of the left inferior, and to a lesser extent the left middle, temporal lobe gyrus. The left superior temporal, fusiform and parahippocampal gyri and the hippocampal formation are notably spared.

Figure 9.8 Voxel compression map time period three (sagittal)



Sagittal MRI of patient 306.IV.3 with voxel-compression-mapping overlay over time period three, showing atrophy of the left operculum, middle and inferior temporal lobes and inferior parietal lobule. The primary motor and sensory cortices, occipital lobe and cerebellum are spared.

10. A volumetric MRI study comparing familial and sporadic Alzheimer's disease

10.1 Background

Although familial AD has an earlier age at onset than sporadic AD, it shares the same neuropathological signature lesions. Some neuropathological studies suggest that some FAD mutations may progress faster than SAD (e.g. Gomez-Isla *et al.* 1999). In this chapter two studies comparing FAD and SAD prospectively are reported to compare the rate of progression in vivo.

10.2 Subjects

10.3.1 Study 1

Thirty two patients with probable AD (NINCDS-ADRDA criteria) and 18 normal controls (NC) were recruited from the Specialist Cognitive Disorders Clinic and the FAD longitudinal study. The AD group comprised of 14 SAD subjects and 18 patients with FAD (six with *APP* and twelve with *PSEN1* mutations. The FAD and SAD patients were severity matched based on their MMSE score at the time of initial MRI. The control patients were selected from the DRG pool of control patients and age and sex matched to the FAD group. All subjects underwent at least two volumetric MRI scans at approximately annual intervals.

10.3.2 Study 2

Twenty two patients with probable AD (NINCDS-ADRDA criteria) were recruited from the Specialist Cognitive Disorders Clinic and the FAD longitudinal study. The study group comprised of 11 SAD subjects (4F:7M) and 11 patients with FAD (5F:6M) from pedigrees with known *APP* or *PSEN1* mutations. All subjects underwent at least three volumetric MRI scans at approximately annual intervals.

10.3 Methods

10.3.1 Study 1

T1 weighted volumetric MRI assessments were performed on the same 1.5 T Signa unit (General Electric, Milwaukee, WI). The individual MRI scans provided 124

contiguous 1.5 mm coronal slices through the head, which were transferred to a Sun workstation (Sun Microsystems Inc., Mountain View, CA) for examination using the MIDAS image analysis programme. The scan pairs were registered by positional matching of the later scan onto the baseline scan. The ventricular cerebrospinal fluid (CSF) space was identified on the earlier scan using a semi-automated technique using manually placed seeds and defined as having a voxel intensity of between 0 and 60% of mean brain voxel intensity. Ventricular volumes included the lateral ventricles and temporal horn of the lateral ventricles but not the third or fourth ventricle. All segmentations were performed by myself; mean intrarater reproducibility was >99.5%. The baseline ventricular CSF volume was then "copied and pasted" onto the later scan where it was edited. This has the advantage that like is compared with like and that the same assumptions to exclude extraneous CSF are made for both sets of images. Further details of the ventricular segmentation protocol are given in Appendix 4.

10.3.2 Study 2

Neuroimaging was performed as described above and the sequential scan pairs were registered by positional matching. The difference in brain volumes was calculated using the brain boundary shift integral (BBSI) as previously described.

10.4 Results

10.4.1 Study 1

The ages (mean ± SD) of the subjects were: FAD = 46.7 ± 7.4 years, NC = 47.9 ± 8.1 years and SAD = 67.3 ± 9.5 years. Their MMSE scores (mean ± SD) at baseline were: FAD = 22.9 ± 6.1, SAD = 19.6 ± 3.9 (NS) and NC = 29.1 ± 0.6 ($P<0.005$). The scan interval (mean ± SD) was 1.60 ± 0.54 years for the FAD subjects, 1.16 ± 0.24 years for the control group and 1.09 ± 0.36 years for the SAD patients. Baseline ventricular volumes (mean ± SD) were: FAD = 24.1 ± 16.8 ml, NC 16.3 ± 10.8 ml and SAD 48.7 ± 28.4 ml. Ventricular volumes (mean ± SD) increased by: FAD = 5.7 ± 4.9 ml/yr, NC = 0.5 ± 0.8 ml/yr and SAD = 6.1 ± 3.2 ml/yr. There were no significant differences between baseline ventricle volumes. The rate of increase in both FAD and SAD ventricle volumes was significantly greater than for NC ($P<0.05$), but there was no significant difference between the FAD and SAD groups.

10.4.2 Study 2

The ages (mean ± SD) of the subjects at their first scan were FAD = 46.5 ± 7.8 and SAD = 64.6 ± 6.7 years. MMSE scores (mean ± SD) were: FAD = 21.8 ± 8.2 and SAD = 20.3 ± 5.2. The BBSI (mean ± SD) for interval one were: FAD = 29.8 ± 14.3 mls and SAD = 14.3 ± 9.8 mls, and for interval two: FAD = 26.4 ± 12.9 mls and SAD = 13.3 ± 5.9 mls. The annualised rates of atrophy (mean ± SD) expressed as a percentage of brain volume at first scan for interval one were: FAD = 2.73 ± 2.58 %/yr, SAD = 2.51 ± 1.15 %/yr (NS). Interval two: FAD = 3.42 ± 2.30 %/yr, SAD = 2.00 ± 1.05 %/yr ($P<0.05$). However, there was one outlier in the FAD group with atrophy rates of 9.94 %/yr and 9.49 %/yr for intervals one and two. These were more than twice as high as the next individual in either group skewing the data. This patient had severe FAD, with a MMSE of 5/30 at baseline and 0/30 at the third scan 12 months later. Excluding this patient reduces the respective FAD rates of atrophy (mean ± SD) to 2.01 ± 1.03 %/yr and 2.81 ± 1.17 %/yr for interval one and two which were not significantly different from those for SAD patients. There were no significant differences in the rates of atrophy between the intervals within the FAD and SAD groups.

10.5 Discussion

Older SAD subjects had larger baseline ventricles than the younger FAD and NC subjects. There was overlap between the groups, especially between FAD and NC and therefore this finding did not reach statistical significance. However, rates of ventricular enlargement in Study 1 were significantly greater in both FAD (5.6ml per year) and SAD (6.1ml per year) compared to NC (0.5ml per year) and the results were similar to those obtained in other recent high resolution MRI which demonstrate a broad consensus (~5-8ml per year) as to the magnitude of ventricular enlargement in AD (see Table 1.2). Discrepancies between these results are likely to be due to protocol differences and inter-subject variation. The changes in AD are substantially higher than those seen in normal ageing. In Study 1, normal controls underwent ventricular change of 0.5ml per year, which compares well with reports from Wang *et al.* (2002) who found 0.4ml per year, Schott *et al.* (2003) who found ventricular expansion of 0.44 ml per year, and Scahill *et al.* (2003) who reported change of

0.65ml per year. Importantly there was no difference in the current study in the rate of change of ventricular CSF volume between the FAD and a similarly severely affected SAD group despite a 20 year difference in age.

Although there is some evidence for more rapid progression in Study 2, when the BBSI is used as a measure of brain atrophy this was entirely due to a single individual with severe FAD (MMSE 5/30) skewing the data. Removal of that outlier resulted in there being no significant difference between the FAD and SAD group. The rate of cerebral atrophy in Study 2 was between 2.0-2.8% per year which is in accord with previous data (see Table 1.2). Furthermore there appeared to be no significant differences between the first and second intervals, in keeping with data from Chapter 6 suggesting that progression in AD is linear over 1-2 years.

11 Conclusions

The data presented in this thesis were collected as part of an ongoing collaborative study involving many people. This thesis addresses several related questions about familial AD. Past genetic screening of the DRG collection of families had identified a causative FAD mutation in only a minority of families. Against this background Chapter 3 reports a mutational analysis of the three known FAD genes. Multiple mutations, both novel and previously reported, were identified. Three different *PSEN1* mutation families are reported in detail in Chapter 4 to assess whether there is a specific *PSEN1* FAD phenotype. Although the clinical features were broadly of early onset AD in all families, family 102 (E280G) additionally had spastic paraparesis and unusual "cotton wool" plaques at neuropathology. With advances in symptomatic AD treatments and the particular relevance of possible amyloid β immunotherapy to FAD families, Chapter 5 is a pilot study which confirms earlier reports of increased plasma amyloid β peptide as a biomarker for FAD. Relatively little is known about the progression of cerebral atrophy using MRI beyond one to two years and whether progression is linear or whether it accelerates - this is addressed in Chapter 6. Based on the neuropathological studies of Braak and Braak (1991) AD is thought to start in the medial temporal lobes. This region's involvement is confirmed *in vivo* in subjects who were initially asymptomatic using volumetric MRI studies in Chapter 7. However, confirming the involvement of the medial temporal lobe early in FAD does not equate to this being the *locus* of disease onset. This is addressed in Chapter 8 which investigates the earliest MRI changes in FAD without *a priori* assumptions using fluid registration. This *in vivo* study showed pre-symptomatic posterior cingulate, neocortical temporo-parietal and medial temporal lobe atrophy which accords well with Braak and Braak (1991). We had the opportunity to also assess the onset of non-AD by prospectively studying a presymptomatic "at risk" family member belonging to a familial FTLD pedigree. Fluid registration revealed very focal left frontal lobe atrophy, clearly different from FAD, which correlated well with the patient's clinical presentation. Relatively little is known about the difference between cerebral atrophy in FAD and sporadic AD patients and this is addressed in Chapter 10. These studies are summarized below using the specific questions stated at the beginning of this thesis in "the problem".

11.1 What proportion of FAD is accounted for by mutations in known genes?

A mutational analysis of the *APP*, *PSEN1* and *PSEN2* genes in 31 probands with probable or definite AD from DRG families with an AAO <61 years is described in Chapter 3. In 21 (68%) probands eight novel *PSEN1* mutatiions, eight previously reported *PSEN1* mutations, a novel *APP* mutation (H677R) and two known *APP* mutations were identified. Nine of the eleven (82%) probands with neuropathologically confirmed AD, who additionally fulfilled recognised criteria for autosomal dominant inheritance, were associated with a sequence variant in *APP* or *PSEN1*. The ten patients, in whom it was not possible to identify a mutation in *APP*, *PSEN1* or *PSEN2,* were over ten years older (P=0.001) than the probands with mutations. No mutations in *PSEN2* were detected. The proportion of *PSEN1* and *APP* mutations described in the present study is much higher than the 18% reported by Cruts *et al.* (1998) who used the same criteria to define autosomal dominant inheritance as in the present study. However, the findings from this study are in accord with a French FAD study in which 24/34 (71%) families fulfilling stricter three generational criteria to define autosomal dominant inheritance were found to have mutations in *APP* or *PSEN1* (Campion *et al.* 1999). The combined post-study DRG FAD mutation prevalence is 88% and the likelihood of a fourth FAD locus existing would appear to be much diminished. However, several large kindreds in the DRG collection of FAD families remain without an identified mutation.

11.2 Is there a specific PSEN1 FAD phenotype?

Three neuropathologically verified *PSEN1* FAD families are described in Chapter 4. The phenotype of family 105/160 (Intron 4) was remarkably similar to previously described *PSEN1* pedigrees and shares their early AAO, the presence of myoclonus and generalised seizures, and a relative preservation of naming skills until late in the disease. Family 177 (L153V) similarly had an early AAO, myoclonus and relatively preserved naming, but no reported generalized seizures. In view of the reduced age at onset in patient 105/160.V.16 and *APOE ε4/ε4* homozygosity, *APOE ε4* may modulate AAO in this pedigree. The neuropathology of family 105/160 and 177 is more severe than sporadic AD and although Lewy bodies were prominent in both

these families their neuropathology is similar to that of other *PSEN1* cases. Family 102 (E280G) however, was associated with significant white matter abnormalities on cranial MRI and/or progressive spastic paraparesis. The characteristic neuropathological findings of diffuse, non-cored, "cotton-wool" amyloid plaques and prominent amyloid angiopathy associated with the FAD spastic paraparesis phenotype were seen in a third affected family member. Neither spastic paraparesis nor significant white matter abnormalities on cranial MRI have been previously described in association with the E280G mutation. Although all three families have an early AAO and broadly have FAD phenotypes there is a remarkable clinical and neuropathological phenotypic heterogeneity in *PSEN1* FAD.

11.3 Is plasma amyloid β peptide a useful biomarker of FAD?

The common mechanism of action for FAD mutations is through modulation of APP processing which alters the synthesis β-amyloid peptides, key components of neuritic plaques. As prospects for the first AD modifying drug improve, the value of a peripheral disease marker with surrogate and pre-symptomatic diagnostic potential becomes increasingly important. Since β-amyloid peptide synthesis occurs in blood platelets as well as the CNS the suitability of plasma β-amyloid peptides as biomarkers of familial AD is described in Chapter 5. Plasma $A\beta_{42}$ concentrations were determined using the highly specific and sensitive Innotest Aβ(1-42) ELISA. Plasma $A\beta_{42}$ concentrations (pg.ml-1) were elevated in both symptomatic (52.8 ± 3.2, $n = 6$; $P<0.02$) and pre-symptomatic (55.2, $n = 3$; $P<0.1$) subjects who possess FAD mutations when compared with asymptomatic family members who did not possess such mutations (29.6, $n = 3$). The mean plasma $A\beta_{42}$ concentration in "at risk subjects" of unknown genotype (37.3 ± 2.2, $n = 11$) was midway between those of FAD mutation carriers ($P<0.01$) and non carriers. These findings are in accord with a previous report (Scheuner *et al.* 1996) and suggest that $A\beta_{42}$ peptide may be a useful biomarker for FAD, even in the absence of symptoms.

11.4 Is the progression of cerebral atrophy in EOAD linear?

In Chapter 6 a longitudinal neuroimaging study is described. Twelve early onset AD patients (nine familial and three sporadic patients) with at least three scans obtained over a period of up to six years were recruited. Brain atrophy was estimated from

sequential brain volume measurements, and also using the brain boundary shift integral (BBSI), a direct estimate of volume loss (between temporally adjacent scan pairs). Ventricular enlargement, an indirect measure of brain atrophy was also assessed. The data were presented relative to the fixed datum of MMSE 23, which relates to mild to moderate disease, and which was reached by all patients during the course of the study. Cerebral atrophy rates appeared approximately linear but were better represented by a quadratic than by a linear relationship with time. When atrophy was derived from sequential measurements of whole brain volume, the estimated increase in rate of atrophy was 0.25% per year (95%CI 0.08 to 0.43). Two years before MMSE 23 cerebral atrophy was estimated at 2.17% of brain volume per year increasing to 3.18% four years later. The values obtained using the BBSI were similar: the estimated increase in rate of atrophy was 0.28% per year (95% CI 0.17 to 0.39), with an estimated rate of atrophy of 1.73% of brain volume per year initially and 2.84% four years later. Ventricular CSF volumes also increased with time from 0.173 % per year (95% CI 0.132 to 0.214) to 0.208 % per year over four years. There was only borderline evidence for acceleration of ventricular enlargement which was estimated at 0.009% per year (95% CI: 0.0000 – 0.0175; P= 0.05), probably because ventricular enlargement reflects only a small proportion of cerebral atrophy. No significant differences were observed between sporadic and familial AD patients, nor was APOE genotype found to affect rate of cerebral atrophy. Brain atrophy rates therefore gradually increase in AD with increasing disease severity, an observation with important implications for the understanding of disease progression in AD, and for the use of brain atrophy as a biomarker of disease severity.

11.5 Is there pre-symptomatic medial temporal lobe atrophy in FAD?

Chapter 7 is a volumetric MRI study of patients with FAD during the period that they developed symptoms. Five patients with presymptomatic FAD (three with *PSEN1* and two with *APP* mutations) and 20 normal controls had two or more annual volumetric MRI brain scans. All five FAD "at risk" subjects subsequently developed probable AD. Volumes of brain, ventricles, temporal lobes, hippocampi, and entorhinal cortices were measured. Rates of volume change were calculated from serial scans. There were no significant differences in baseline measures of whole brain, temporal lobe, or ventricular volume between patients and controls. However, baseline averaged

volumes of medial temporal lobe structures (hippocampi and entorhinal cortices) were 16.6% (95% confidence interval [CI], 3.3-28.0%) lower in the patient group. Atrophy rates for brain, temporal lobe, hippocampus, and entorhinal cortex were significantly increased in patients compared with controls ($p < 0.05$). Averaged atrophy rates from both hippocampi and entorhinal cortices were 5.1% (95% CI, 3.0-7.1%) greater in patients than controls. Linear extrapolation backward suggested medial temporal lobe atrophy commenced 3.5 years (95% CI, 0.7-7.5 years) prior to the commencement of the study, when all patients were asymptomatic. In conclusion increased medial temporal lobe atrophy rates are an early and distinguishing feature of FAD and pathological atrophy is probably occurring several years before the onset of symptoms in FAD.

11.6 Where does cerebral atrophy in FAD begin?

This question is addressed in Chapter 8 using "fluid" registration, a technique that treats the brain as a viscous liquid rather than a rigid body. It addresses the criticisms generally levelled at volumetric imaging studies where *a priori* assumptions are made about areas involved in AD neurodegeneration. Serial imaging was acquired in four asymptomatic "at risk" individuals from FAD families (families 19, 105/160, and 148, respectively carrying the causative *APP* V717G, *PSEN1* intron 4 and *PSEN1* M139V mutations). All four subjects became symptomatic over a period of five to eight years during research follow-up. In addition 20 individuals with a clinical diagnosis of probable AD and 20 control subjects underwent serial brain MRI. A non-linear fluid matching algorithm was applied to register repeat scans onto baseline imaging. Jacobian determinants were used to create a voxel compression map, allowing localisation of volume change in an individual over time. Using fluid registration progressive atrophy was revealed in presymptomatic "at risk" FAD individuals with posterior cingulate and neocortical temporo-parietal cortical losses as well as medial temporal lobe atrophy. In established cases atrophy was widespread but spared the primary motor and sensory cortices and cerebellum, reflecting the clinical phenomenology. Voxel compression maps confirmed early involvement of the medial temporal lobes but also showed posterior cingulate and temporo-parietal cortical losses at the *pre*symptomatic stage. This distribution of atrophy in both preclinical and established AD accords well with the pattern of cognitive deficits and the

distribution of observed histological changes at autopsy (Braak & Braak 1991). If Braak & Braak's (1991) cross-sectional neuropathological data are viewed as a continuum the locus of initiation of Alzheimer's disease can be considered to be in the medial temporal lobe. The voxel compression mapping data provide longitudinal data compatible with this. However, this study cannot determine the stage at which structural changes are first present, only that atrophy rates increased several years before symptom onset. Similarly this study cannot address the issue of when histological changes (plaques, tangles and cell loss) start but it seems reasonable to suggest that they are likely to precede the macroscopic, atrophic changes demonstrated.

11.7 Is the onset and progression of cerebral atrophy different in non-AD dementia?

Fluid registration was also applied in Chapter 9 to demonstrate the onset and progressive pattern of *in vivo* atrophy in familial FTLD. An asymptomatic "at risk" family member of DRG family 306, a neuropathologically confirmed FTLD family with ubiquitin positive and tau negative inclusion bodies, was recruited for a longitudinal clinical, neuropsychological and neuroimaging study. The patient was an asymptomatic 51 year old woman studied over a period of 69 months with approximately annual clinical, MRI and neuropsychological assessments. Over the first 26 months of the study, she remained asymptomatic, but subsequently developed progressive speech production difficulties, and latterly severe orofacial dyspraxia, dyscalculia, frontal executive impairment and limb dyspraxia. Regional atrophy was present prior to the onset of symptoms, and was initially centred on the left dorsolateral prefrontal cortex and the left middle frontal gyrus. Latterly, there was increasing asymmetric left frontal and parietal atrophy. Medial temporal lobe structures, the sensorimotor cortices and the cerebellum were unaffected. Neuropsychological evaluation revealed mild intellectual impairment prior to the onset of these clinical symptoms; frontal executive and left parietal impairment subsequently emerged, culminating in widespread cognitive impairment. Fluid registered MRI allowed the emerging atrophy patterns to be delineated and the neuropsychological and clinical data to be correlated with structural change. Fluid registration therefore revealed initially very focal left frontal lobe atrophy, clearly

different from FAD, which correlated well with the patient's clinical presentation.

11.8 Does cerebral atrophy in FAD progress faster compared with SAD?

Early onset AD, and FAD in particular, is generally believed to be more severe than SAD. Chapter 10 consists of two studies comparing rates of atrophy between FAD and SAD. The first study compares change in ventricular CSF volumes, whereas the second study looks at change in total brain volume using the BBSI. Older SAD subjects had larger baseline ventricles than the younger FAD and control subjects. There was overlap between the groups especially between FAD and control subjects. However, rates of ventricular enlargement were significantly greater in both FAD and SAD compared to controls. Importantly there was no difference in the rate of change between the FAD and a similarly severely affected SAD group despite a 20 year difference in age. Although there is some evidence for more rapid progression in the second study when the BBSI is used as a measure of brain atrophy this was entirely due to a single individual with severe FAD (MMSE = 5/30 at first scan) skewing the data. Removal of that outlier resulted in there being no significant difference between the FAD and SAD group. On balance there appears to be no difference in the progression of cerebral atrophy in FAD and SAD.

11.9 Implication of findings and possible future studies

The majority of DRG FAD families now have an identified mutation. A number of mutations were found in families that had previously been screened, suggesting that the mutations were outside the sequenced areas. This is unlikely to be an isolated finding and historical series in the literature may therefore underestimate the proportion of FAD pedigrees accounted for by the known FAD genes. Although the whole of *PSEN1* was sequenced, only specific exons of *PSEN2* and *APP* were analysed. In the first instance the sequencing of the remainder of *PSEN2* is planned, but it may be that *APP* needs to be looked at again. Furthermore the families that remain without mutations will need careful review of both the clinical and neuropathological features, before considering other genetic (single gene or multifactorial) or alternative explanations.

The characterisation of the *PSEN1* phenotype(s) has revealed a number of similarities, but also marked heterogeneity. In family 105 the patient with the youngest AAO was *APOE ε4/ε4* homozygous. Whether *APOE ε4* modulates AAO in this pedigree is worthy of further study. Lewy body pathology was prominent in families 105/160 and 177. It is unclear how APP processing influences Lewy body pathology, but this is an increasingly recognised finding in FAD. There may be an interaction between α synuclein and APP, which may have relevance for AD, dementia with Lewy bodies and Parkinson's disease dementia. The presence of spastic paraparesis in family 102 is also of interest. Family 102 is a large pedigree and the current "at risk" generation is involved in the DRG longitudinal study, which may shed more light on the factors that determine the absence/presence of spastic paraparesis. Review of the available neuropathology to see whether cotton wool plaques are present in the absence of reported paraparesis would also be important. Since families 168 and 183 also carry the *PSEN1* E280G mutation it would be of interest to review their phenotype and any available brain bank tissue for similar reasons.

The small pilot study of plasma $A\beta_{42}$ as a possible FAD biomarker was designed when FAD mutations were generally thought to result in the increased synthesis of the more amyloidogenic $A\beta_{42}$. There are now a number of important exceptions to this general principle. However, if a specific FAD mutation's effect on APP processing is known, then plasma Aβ measurements can be interpreted appropriately. There may therefore be a limited role for plasma Aβ as a biomarker, possibly for assessment of the *in vivo* effects of potential disease modifying agents in cultured media, animal models or even human FAD patients.

A number of important findings where described in the imaging chapters. Increased pre-symptomatic medial temporal lobe atrophy was demonstrated in FAD patients when compared to matched control patients. Baseline FAD medial temporal lobe volumes were found to be lower than in control patients suggesting that atrophy started several years before symptom onset. Non-linear (fluid) registration was used to determine the area where cerebral atrophy starts without *a priori* assumptions about the areas involved in AD neurodegeneration. Posterior cingulate and neocortical temporo-parietal cortical losses as well as medial temporal lobe atrophy were the earliest changes identified. In established cases atrophy was widespread but spared the

primary motor and sensory cortices and cerebellum. This data provides important biological validity for the registration technique. The rate of cerebral atrophy was found to accelerate with increasing disease severity. Equally important was the fact that the rate of atrophy was linear over one to two years, the time period used in typical drug trials. Whole brain atrophy was a better measure of cerebral atrophy than ventricular volumes, almost certainly because of their relative sizes. The acceleration of cerebral atrophy could indicate true acceleration of atrophy in already affected brain regions, disease spread to a greater proportion of cerebral cortex, or both. This question could be addressed with non-linear (fluid) registration. Acceleration of disease progression would suggest that for a given drug trial design more statistical power could be achieved by the enrolment of more severely affected patients. Conversely, over short periods atrophy was linear suggesting that this variable does not need to be controlled for over one to two years, and attenuation of cerebral atrophy could be due to a treatment effect. Although the imaging studies described in this thesis cannot determine when neuropathological or even structural changes first occur, the findings accord well with the clinical phenomenology and Braak & Braak's (cross-sectional) neuropathological model (Braak & Braak 1991). It seems reasonable to assume that the neuropathological changes predate tissue loss, and therefore measurable cerebral atrophy. It then follows that there is significant pre-symptomatic disease activity. A comparison of rates of cerebral atrophy between FAD and SAD patients showed no difference in the progression of these groups. This needs to be looked at in more detail, before concluding that the FAD findings have broader implications. Similarly the detailed study of familial FTLD, in which very different clinical and imaging changes from FAD were described, may not immediately be applicable to sporadic FTD. However, similar conclusions about pre-symptomatic changes can be drawn to those listed for FAD. The potential of the registration technique(s) would benefit assessment in different dementias. Should specific dementia disease modifying drugs become available, early and accurate diagnosis will become increasingly important. Serial imaging demonstrating excess atrophy above that expected for age may be a useful method of detecting early neurodegeneration and the pattern of regional atrophy may prove to be a useful tool in the differential diagnosis of degenerative dementias. However, the validation of cerebral atrophy as a true surrogate marker of AD (and degenerative dementia in general) must await the development of a true disease modifying agent.

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Appendix 1: Tau (MAPT) gene mutation phenotypes

Abbreviations used in table:

3R = three repeat tau

4R = four repeat tau

CBD = corticobasal degeneration

DDPAC = disinhibition dementia parkinsonism amyotrophy complex

DRG = Dementia Research Group 10^{+16} families (Janssen *et al.* 2002)

F = frontal

FTD = frontotemporal dementia

MAAD = mean age at death

MAAO = mean age at onset

MND = motor neurone disease

MSTD = familial multi system tauopathy with presenile dementia

MT = microtubule

NFT = neurofibrillary tangles

PA = progressive nonfluent aphasia

PHF = paired helical filaments

PPND = pallido-ponto-nigral degeneration

PSG = familial progressive subcortical gliosis

PSP = progressive supranuclear palsy

T = temporal

Mutation	Mechanism	Clinical	MAAO (range) yrs	MAAD (range) yrs	Duration (yrs)	Neuropathology	Tau filament morphology	First Author
K257T	All tau isoforms increased, but mainly 3R; reduced MT assembly	FTD in proband with late semantic memory impairment. Not clearly autosomal dominant.	47	51	4	F<T atrophy. Tau immunoreactive Pick bodies in neocortex, hippocampus and subcortical areas.	Narrow irregularly twisted filaments	Rizzini, 2000
G272V	All tau isoforms increased. Reduced MT assembly	HFTD2 family: FTD	46.5	54.7	8.5 (4-16)	F-T atrophy. Ballooned cells in cortex and basal ganglia, Tau positive inclusions in multiple cortical and subcortical areas and in dentate gyrus	-	Hutton, 1998; Heutink, 1997; Spillantini, 1998a; Hasegawa, 1998;
10^{+3}	Increased 4R tau; enhanced exon 10 splicing	MSTD family: disequilibrium, short term memory, progressive cognitive decline, extrapyramidal syndrome, vertical gaze palsy and dysphagia.	49 (39-59)	-	10	Diffuse atrophy of neocortex, subcortical nuclei, brainstem, and spinal cord. Neuronal and glial tau inclusions	Twisted ribbons	Spillantini, 1997 & 1998b; Murrell, 1997
10^{+12}	Increased 4R tau; enhanced exon 10 splicing	Kumamoto pedigree: FTD and parkinsonism	53	-	7	F-T atrophy. Neuronal and glial tau inclusions	Twisted ribbons	Yasuda, 2000
10^{+13}	Increased 4R tau; enhanced exon 10 splicing	Man19 family: FTD	65	-	-	-	-	Hutton, 1998
10^{+14}	Increased 4R tau; enhanced exon 10 splicing	Family Mo (DDAPC): disinhibition, hyperphagia, early memory loss, anomia, constructional apraxia, extrapyramidal syndrome,	45 (27-56)	57 (35-69)	13 (5-23)	F-T atrophy. Depigmentation of substantia nigra. Ballooned neurones.	-	Hutton, 1998; Lynch, 1994
10^{+16}	Increased 4R tau; enhanced exon 10 splicing	Families: Aus1, PSG-1 (FPSG, A), FTD002, Man6, Man23, EKR, and DRG all FTD	Aus1: 53 (39-64); PSG-1: 49 (41-58); EKR: 48 (48-55), DRG 50 (37-59)	Aus1: 62 (49-69); PSG-1: 60 (54-69); EKR 65 (65-75); DRG 61 (42-72)	4-14; 7-17; DRG 11(3-22)	F-T atrophy. Neuronal and glial tau pathology in PSG-1 and DRG	PSG-1: twisted ribbons	Hutton, 1998; Goedert, 1999a; Dark, 1997;Poorkaj, 2001; Janssen, 2002
N279K	All tau isoforms increased, but tau positive inclusions are mainly 4R; enhanced exon 10 splicing	PPND family: progressive parkinsonism with dystonia, personality change, dementia, ocular motility abnormalities, pyramidal tract dysfunction, frontal lobe release signs	43 (32-52)	52 (46-63)	9 (5-19)	Mild F-T atrophy. Depigmentation of substantia nigra, pallidum and caudate. Widespread neuronal and glial fibrillary tangles.	Twisted and straight filaments.	Clark, 1998; D'Souza, 1999; Reed, 1998; Hasegawa, 1999; Hong, 1998; Wszolek, 1992
delK280	Effect on exon 10 uncertain; reduced MT assembly	Demonstrated in a Dutch FTD patient whose father had been diagnosed with Parkinson's disease	-	-	-	-	-	D'Souza, 1999; Rizzu, 1999

Mutation	Mechanism	Clinical	MAAO (range) yrs	MAAD (range) yrs	Duration (yrs)	Neuropathology	Tau filament morphology	First Author
L284L	Increased 4R tau; enhanced exon 10 splicing	LKL pedigree: FTD but, early visuospatial impairment in proband	52 (47-61)	62 (57-71)	10 (9-10)	Symmetrical F>T atrophy. Amyloid and tau deposition		D'Souza, 1999
N296N	Increased 4R tau; enhanced exon 10 splicing	FTD in proband with late supranuclear gaze palsy, orofacial dyspraxia and bradykinesia	56	69	13	Symmetrical F-T atrophy. Swollen achromatic neurons and corticobasal inclusion bodies. Glial and neuronal tau positive inclusions.	-	Spillantini, 2000; Brown, 1996
P301L	All tau isoforms increased; reduced MT assembly	HFTD1 family and Seattle families D, F, & G: FTD in all families. Variation in AAO in Seattle families not due to ApoE. Family MN: FTD and PA phenotypes.	HFTD1: 50.4; D: 49 (41-57); F: 61 (56-67); G: 64 (57-75); MN: 58 (55-63)	HFTD1: 58.6; D: 56 (46-63); F: 68 (56-77); G: 72 (65-80); MN: 65 (62-68)	HFTD1: 8.5 (4-16); D: 6 (5-6); F: 8 (4-13); G: 7 (5-11); MN: 7 (5-12)	Severe F-T atrophy. Ballooned cells, degeneration of substantia nigra. Glial and neuronal tau positive inclusions. Seatle D: circular NFT in one patient, brainstem NFT similar to PSP in the other. Family MN: one patient had MND with Pick like bodies	Irregularly twisted ribbons	Hutton, 1998; Heutink, 1997; Spillantini, 1998a; Hasegawa, 1998; Nacharaju, 1999; Bird, 1999; Nasreddine, 1999
P301S	All tau isoforms increased; reduced MT assembly	Family P: FTD (n=1) and CBD (n=1); German family: FTD, parkinsonism and epilepsy in late stage	Family P: 28 (27-29); 20s	36; 35 (24-46)	7	F-T atrophy. Extensive filamentous pathology of hyperphosphorylated tau	Straight filaments	Bugiani, 1999; Goedert 1999b; Sperfeld, 1999
S305N ($=10^{-2}$)	All tau isoforms increased, but inclusions are mainly 4R; enhanced exon 10 splicing	Japanese family with FTD; minimal parkinsonism in 1/3 patients	35 (29-38)	40 (35-41)	5 (3-6)	F-T atrophy: Glial and neuronal tau positive inclusions. Unusual ring shaped NFT.	Straight tubules	D'Souza, 1999; Hasegawa, 1999; Iijima, 1999
S305S	Enhanced splicing of exon 10	frontal dysexecutive symptoms, progressive dementia, limb dystonia, parkinsonism, dysarthria, and supranuclear gaze palsy	51 (48-55)	54 (51-56)	6 (4-7)	NFT concentrated in basal ganglia, gliosis, tufted astrocytes, ballooned neurones. Fulfils criteria for PSP	Twisted and straight filaments.	Stanford, 2000
V337M	All tau isoforms increased. Reduced MT assembly	Early antisocial psychotic symptoms; subsequent confusion and progressive memory loss; dementia	53 (42-66)	67 (55-78)	15 (4-26)	F-T atrophy. NFT in multiple cortical areas. Degeneration of amygdala. Glial and neuronal tau positive inclusions. No neuritic plaques	PHF	Hasegawa, 1998; Poorkaj, 1998; Sumi, 1992; Spillantini, 1996
E342V	Increased 4R tau	Proband FTD and late parkinsonism	44 (39-48)	50 (45-55)	7 (6-7)	F-T atrophy. Glial and neuronal tau positive inclusions. Pick bodies (especially in CA1) and Pick cells.	PHF	Lippa, 2000
G389R	All tau isoforms increased; reduced MT assembly; no effect on exon 10 splicing.	Family F: PA; case 1 FTD with parkinsonism. Two separate mutations: G->C and G->A respectively. Not clearly autosomal dominant.	38;32	43;37	5;5	F>T atrophy. Severe neuronal loss, astrocytosis, marked tissue vacuolation. Pick like inclusions (also in dentate fascia). Tau positive "coiled bodies" in WM. Increased susceptibility of tau to calpain I digestion	Case 1: straight and twisted filaments	Murrell, 1999; Pickering-Brown, 2000
R406W	All tau isoforms increased. Reduced MT assembly	Iowa kindred (Danish origin): FTD, some individuals with parkinsonism and gaze disturbance	55 (45-75)	73 (70-76)	26 (20-35)	Mild F-T atrophy. Severe hippocampal atrophy with abundant NFT. Depigmentation of substantia nigra with abundant NFT. No neuritic placques. Neuronal tau positive inclusions. Fulfils criteria for PSP.	PHF	Hutton, 1998; Hasegawa, 1998; Reed, 1997

Appendix 2: Standardised MMSE

Modified from Folstein *et al.* (1975)

What is the Year ? (exact) __(1)
What is the season of the year? (-1week, +2weeks) __(1)
What is the date? (±1 day) __(1)
What is the day of the week? (exact) __(1)
What is the month? (exact) __(1)
What country are we in? __(1)
What county or city are we in? __(1)
What town or area are we in? __(1)
What is the name of this place? __(1)
What floor of the building are we on? __(1)

I am going to name three objects. After I have said them, I want you to repeat them. Remember what they are because I am going to ask you to name them again in a few minutes.

BUS DOOR ROSE

Record number of trials: _____ (Score 1st trial only) __(3)

Please start with 100 and count backwards, subtracting 7 each time, go on to spelling of WORLD unless serial 7's are **all** correct: *93,86,79,72,65*:

___ ___ ___ ___ ___ *(__(5))*

What were the three objects I asked you to remember?

BUS DOOR ROSE __(3)

I am going to give you a word and ask you to spell it forwards and backwards. The word is WORLD. First spell it forwards?. Now spell it backwards.

___ ___ ___ ___ ___ *(__(5))*

Show a wrist watch. What is this called? __(1)
Show a pencil. What is this called? __(1)
Repeat after me, exactly as I say it: "No ifs ands or buts" __(1)
I'm going to give you a piece of paper. When I do, take it in your right hand, fold the paper in half with both hands, and put the paper on the floor. __(3)
Read the words on the page, then do what it says. CLOSE YOUR EYES __(1)
Write a complete sentence on the piece of paper for me. __(1)
Here is a drawing. Please copy the drawing as accurately as you can. __(1)

TOTAL __(30)

Spring: 1st Mar-31st May, **Summer:** 1st Jun-31st Aug, **Autumn:** 1st Sep-30th Nov, **Winter:** 1st Dec-28th Feb

NHQS: *Queen Square, Holborne, Russell Square, The City.* **St Mary's:** *Paddington, Edgeware Rd, Marylebone*

Serial 7's: Each subtraction may be prompted eg. 93-7. Score mathematical correctness ie 92-7=85. Allow 10-20 secs per reply and stop after 2 incorrect answers. Unless all 5 are correct, go on to WORLD backwards.

CLOSE YOUR EYES

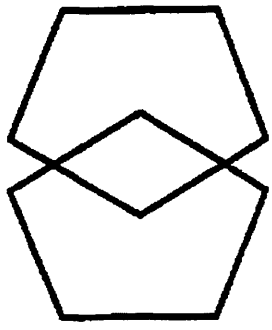
Please write your sentence in the space below:

.....

.....

.....

Please copy this diagram on the bottom half of this page



Appendix 3: Semi-automated labelling of brain regions

The aim of the procedure is to label all brain voxels while excluding non-brain, for example, CSF or dura. The procedure incorporates conditional morphology procedure and makes use of the knowledge that the brain is the largest structure in a 3D scan of the head and that it is only weakly connected to adjacent structures, (e.g. the optic nerve is a weak connection to the eye). In most cases whole brain segmentation can be accomplished by four steps: thresholding, erosion, dilation and internal rethresholding.

Step 1 - Intensity Thresholding

Intensity thresholds are set to exclude voxels brighter than brain (e.g. scalp) and darker than brain (e.g. CSF). The upper threshold is set first. Anything brighter than the upper threshold will be excluded. The aim is chose thresholds to exclude most tissues brighter than white matter including susceptibility artefact (esp. inferior temporal lobe) while not excluding normal white matter.

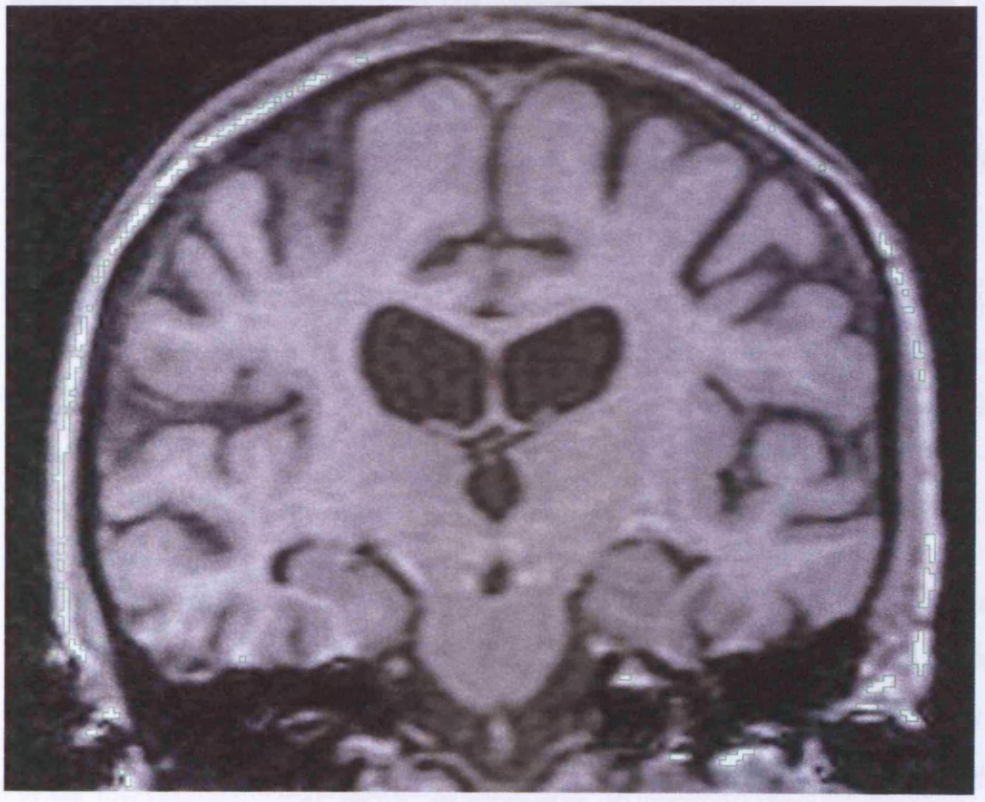


Figure A3.1: Initial thresholding with lower threshold = 0, upper threshold set to exclude high signal (most of scalp).

The lower threshold is set to include as much of brain as possible while minimising inclusion of non-brain. This threshold is typically half the value of the upper threshold. The green intensity region should now include most of the brain.

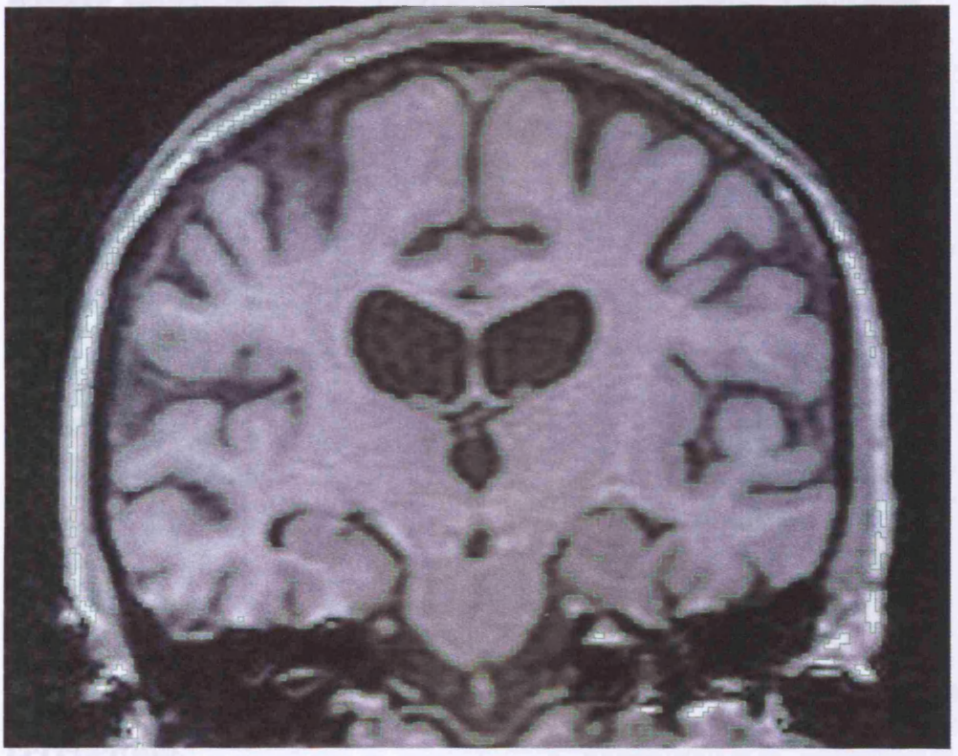


Figure A3.2: Upper and lower thresholds set to include brain and exclude CSF

Ideally the lower threshold should be taken to a high enough value so that in the next step only one erosion is needed to remove non-brain and leave only brain. This requires the thresholding step to have reduced the size of "bridges" between brain and scalp.

The final part of this step is to set the axial cut off slice. This removes any structures below the inferior edge of the cerebellum. This is set when viewing the image in the coronal plane - removing all axial slices below the lowest extent of the cerebellum. Once this step is completed and ***Accept*** clicked Midas searches for the largest connected 3-D object in the current region (i.e. brain) and removes any smaller objects.

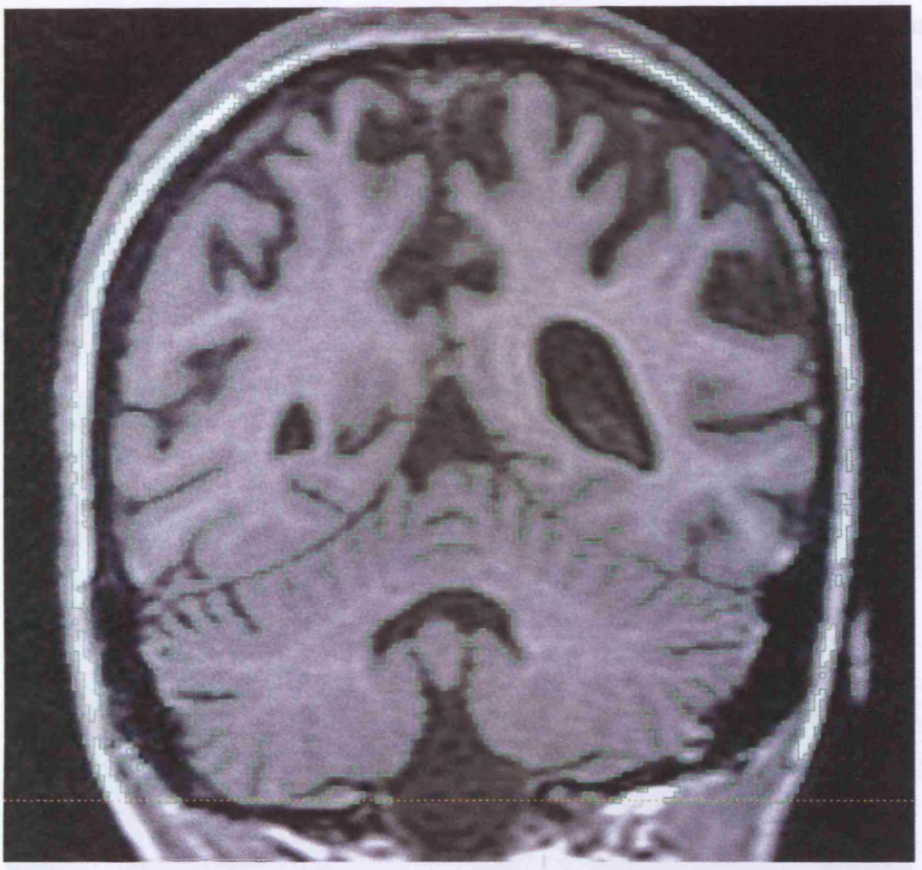


Figure A3.3: The axial cut-off should coincide with the bottom of the cerebellum.

Step 2 - Erosion

The erosion removes edge-voxels from all surfaces of the region. The purpose is to break thin connections between brain and scalp. After each erosion the program retains only the largest connected region. The minimum erosions necessary to isolate brain should be done. The usual range of erosions is [0, 3]. High numbers of erosions remove significant quantities of brain from the region which cannot be recovered in the Dilation stage. The erosions are conditional in that a range of signal intensities which determines which voxels can be eroded - for instance setting an upper threshold at the intensity of white matter will only erode voxels with a signal intensity beneath this value. The number of erosions required are increased one at a time. Often the brain remains connected to scalp on only a few slices, often around the temporal lobes. If connections can be identified visually then they can be broken interactively at this stage by striking through them with the mouse while holding

down the middle button. When all connections between brain and non-brain have been removed, all parts of the region external to brain are removed automatically.

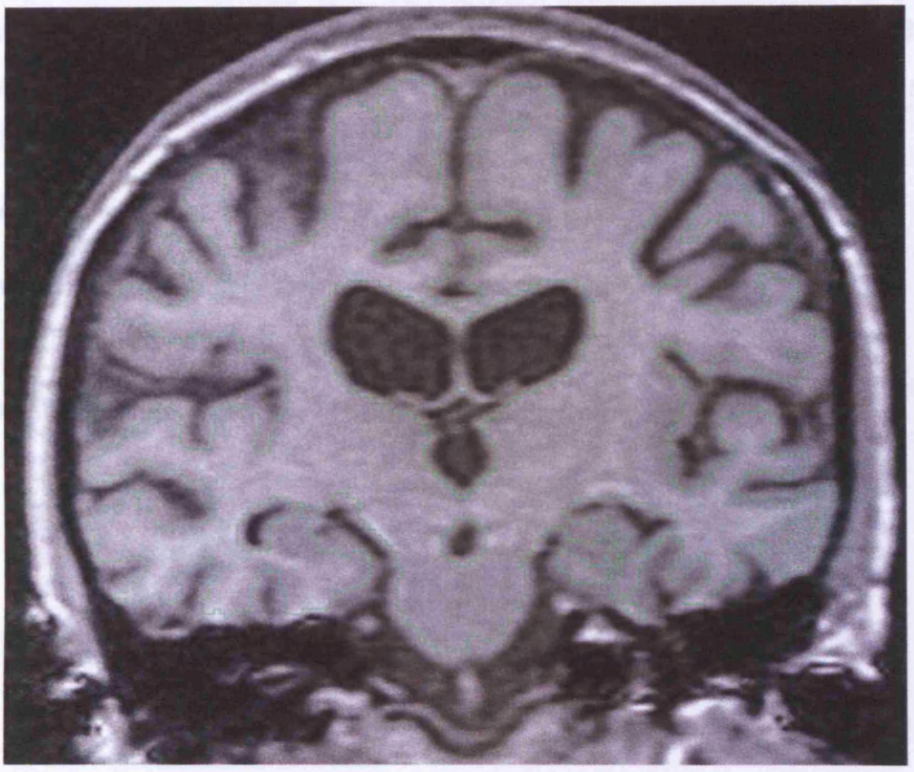


Figure A3.4: An eroded brain region disconnected from surrounding tissue.

Step 3 - Conditional Dilation

The purpose of this step is to recover the brain voxels that has been removed by erosion. Each dilation grows out from the edges of the current brain region but only into voxels within defined range of intensities which represent brain.. The intensity thresholds that appear at this point are automatically set by calculating the mean signal intensity over the whole brain region after step 2 and taking 60% and 160% of this value. These percentages have been determined to be suitable for T1-weighted volumes. The thresholds are re-calculated after each dilation as more voxels are recovered. The number of dilations that are needed to recover all brain voxels depends on the number of erosions (typically = number of erosions + 2).

Step 4 - Rethresholding

This step reclaims remaining excluded areas and completes the segmentation. The rethresholding box is a box of X^3 voxels that moves across the outlined brain region and fills in any area within that region that has been omitted by step 3 subject to the following constraints:

- the omitted area must be smaller than the box.
- only those voxels that have a signal intensity of between 60% and 160% of the mean brain intensity will be included.
- the missing area must already be internal to the region.

Appendix 4: Ventricular segmentation

The aim of this protocol is to label areas of ventricular cerebrospinal fluid taking care not to include extraneous CSF spaces. Before the process can begin ensure that the corresponding brain region is loaded from the brain database and that the coronal view can be seen in the MIDAS window.

Step 1* - *Preparing the image

1. The initial step is to calculate the upper threshold to be used in the segmentation process. This is done by highlighting the brain region and choosing *Measure :Simple Mean*. This calculates the mean voxel intensity over the whole region and computes 60% and 160% of the mean. The values can be seen in the *Log* window. Make a note of the 60% value.
2. The next step is the removal of the outlined region. This is achieved by clicking on *Regions* followed by *Remove*. This is the end of the preparation process.

Step 2* - *Setting the threshold

1. The segmentation tool required for this procedure is the *Irregular Volume* window which is found in *Regions*: *Edit: Irregular Volume*. Once the window is open line up the MIDAS and *Irregular Volume* windows side by side so that the images in both windows can be viewed simultaneously. In the *Irregular Volume* window choose the sagittal view. Set the brightness in the MIDAS window to a satisfactory level and increase the magnification of both images until the ventricles can be clearly seen.
2. To set the threshold click on *Threshold* in the *Irregular Volume* window toolbar and move the Upper threshold slide bar to the 60% value. The lower threshold remains at zero. This ensures that any voxel with an intensity of above 60% of the mean will be excluded (which eliminates all white and grey matter) but ensures that all CSF areas are included.

Step 3* - *Segmentation

1. Set the starting point by moving the dotted position bar in the MIDAS window so that it lies midway across the left lateral ventricle. In the *Irregular Volume* window (which is now showing the corresponding slice in the sagittal plane)

move the dotted bar anteriorly to a point approximately one third of the ventricle length. The image in the MIDAS window will change to the corresponding coronal slice.

2. In the *Irregular Volume* window toolbar click on *Seed* and, in the MIDAS window image, put a seed in the centre of each of the lateral ventricles (achieved by clicking on the right mouse button). The area will be highlighted in both the coronal and sagittal images. If there are no CSF spaces in the temporal lobes (temporal horn) move up one slice. The seeds will be automatically retained. If the seed has missed the ventricle, place another seed in the centre and the missing area will be highlighted. Continue moving up each slice until the lateral ventricles are totally separated from any other CSF areas. When this point is reached click on *Pgt Up* in the *Irregular Volume* window toolbar and *Continue* when the warning box appears. This will propagate the seeds up through every slice anterior to the present slice and highlights the ventricle. Make sure this is only used when the ventricles are not joined to any other CSF spaces as propagating will result in all adjoining CSF being highlighted. If this does occur, the propagation can be cancelled by clicking on *Wipe* + in the *Irregular Volume* window toolbar. To check the propagation, move the dotted position bar on the *Irregular Volume* image to a couple of points anterior to the highlighted edge of the ventricle.
3. Occasionally, the temporal horn will be in view when the procedure is started. If this is the case seeds need to be placed in these spaces. The more anterior the slice the smaller the temporal horn will become until it is no longer visible.
4. When all slices anterior to the starting slice appear to be highlighted it is time to move posteriorly. Take the dotted position bar on the *Irregular Volume* window to the starting position (the first highlighted area). Move down a slice at a time. As before, the seeds will be automatically retained. In contrast to point 3, the more posterior the slice the more of the temporal horn will be in view. Care needs to be taken when highlighting this area as extraneous CSF spaces can often be included. To ensure consistency, follow this rule: As you look at either the left or right hippocampus in the coronal view, imagine a line following the curve of the relative parahippocampal gyrus and then extending up into the hemisphere (avoiding the hippocampus) running almost parallel to the corresponding ventricle. Any CSF spaces to the left (if viewing the right hippocampus) or right

(if viewing the left hippocampus) of this line should be included whereas any spaces to the right/left respectively will be excluded (see examples Fig A4.1 and Fig A4.2).

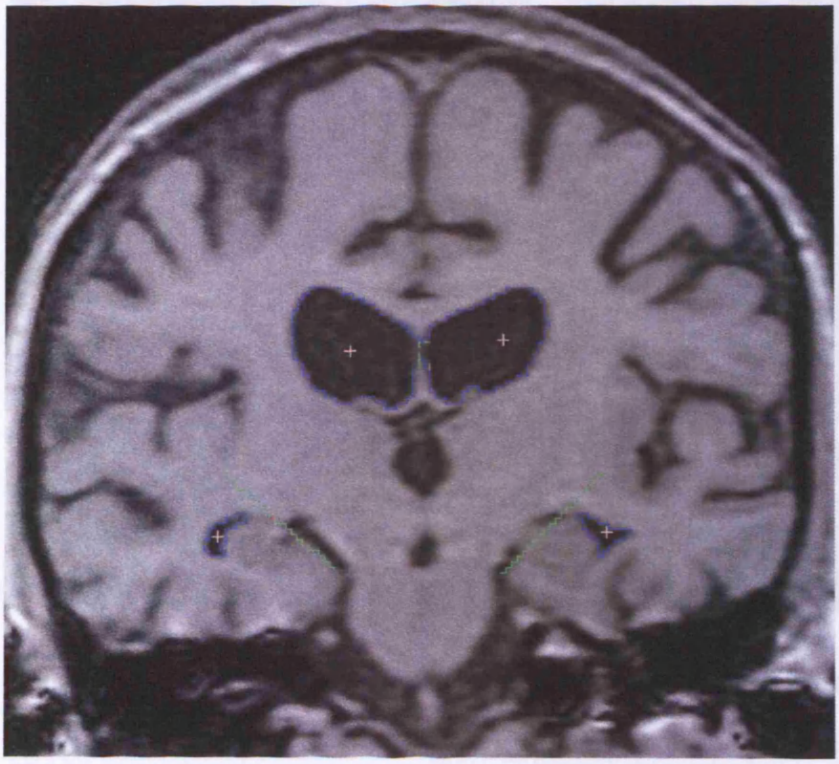


Figure A4.1 Temporal horn of lateral ventricles manual border shown (i)

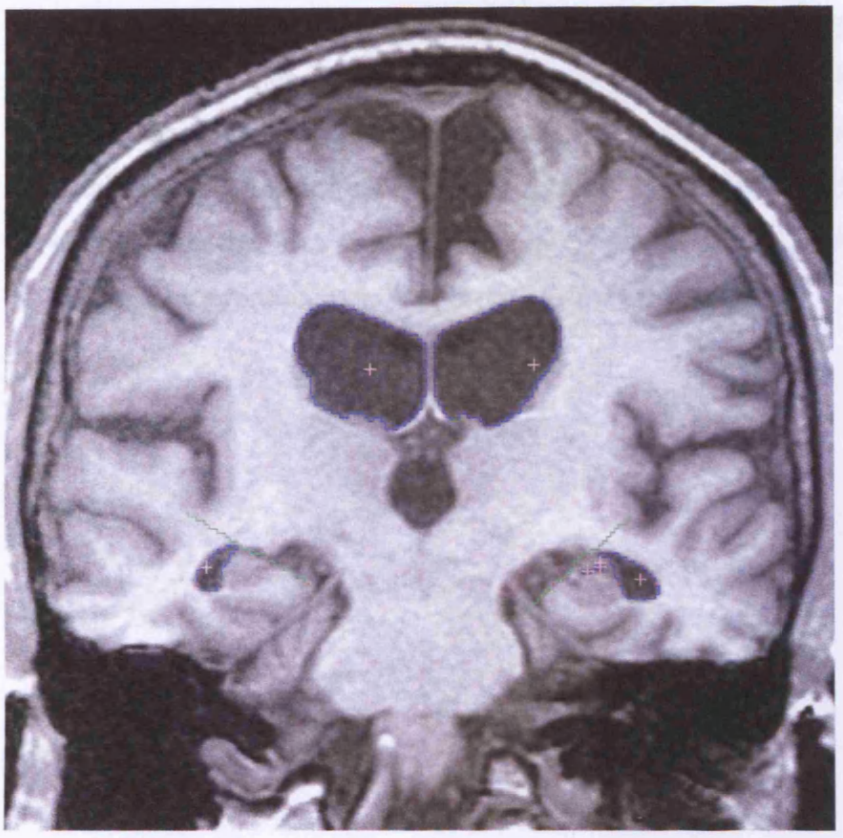


Figure A4.2 Temporal horn of lateral ventricles manual border shown (ii))

5. Moving posteriorly the lateral ventricles and temporal horn will begin to converge. As the CSF areas start to appear use as many seeds as possible to ensure that all CSF is highlighted. Some apparently dark areas will not be included as their voxel intensity is above the 60% threshold. Continue in this manner until a slice is reached where no ventricle spaces are seen. Again care needs to be taken as some subjects have a single hemisphere lateral ventricle that extends far beyond the ventricle in the opposite hemisphere. This area has to be included. In cases of uncertainty, check the corresponding slice on the *Irregular Volume* sagittal or axial image using the position bar.
6. Once all slices appear to have been segmented move the coronal image to approximately slice 65 or any other central slice where lateral ventricles and temporal horn can be seen. Using the position bar, check around each lateral ventricle and each temporal horn on both the coronal and sagittal planes to ensure that no slice has been missed and no extraneous CSF areas have been included. Seeds (unwanted areas) can be removed by clicking the middle mouse button on

the seed thereby removing it and then *Clean*. Repeat looking at the coronal and axial planes. When the segmentation process is complete, click *OK*.

7. To database the segmented ventricle region, make sure that the ventricle regional database is open (*Regions - Show Database - Ventricles)*. Highlight the region by clicking on the blue outline, click on *Regions* then *Database* and enter the required information including the 60% threshold value.

Electronic Database Information

URLs for the data in this thesis are as follows:

- GenBank: http://www.ncbi.nlm.nih.gov/Genbank/Genbank Overview.html
- Online Mendelian Inheritance in Man (OMIM): http://www.ncbi.nlm.nih.gov/Omim/
- Online Alzheimer Disease & Frontotemporal Dementia Mutation Database: http://www.molgen.ua.ac.be/ADMutations

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